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ABSTRACTS

2020 CIS Annual Meeting: Immune Deficiency & Dysregulation North American Conference

(2) Submission ID#755555

Unexpected Oral Lesions in a Patient with a Novel Cytotoxic T-lymphocyte Antigen-4 (CTLA-4) Variant - A Case Report

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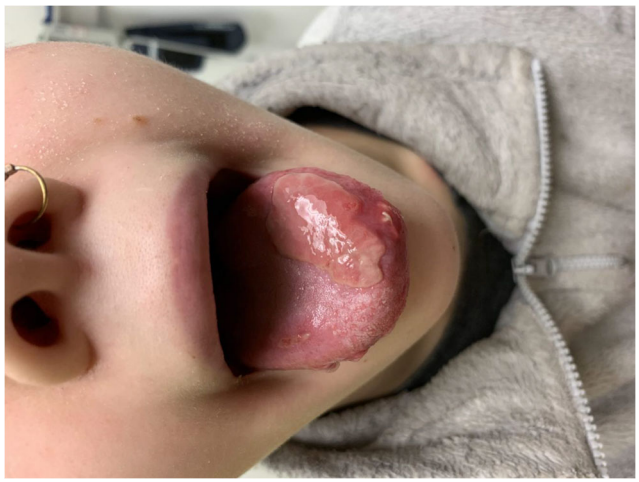
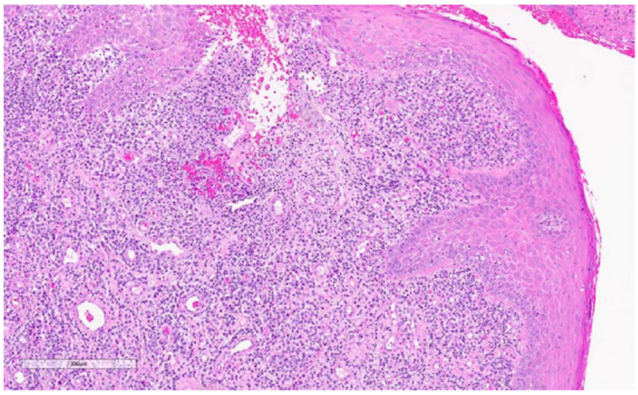
Abstract/Case Report Text
Oral lichen planus (OLP) is a T-cell mediated chronic inflammatory tissue reaction in which presentation can range from asymptomatic plaques to painful, erosive, bullous, or ulcerative lesions. Here, we present a 15 year-old female with a novel CTLA-4 variant, multiple autoimmune conditions, and unusual tongue lesions. Our patient was healthy until 9 years of age when she developed Hashimoto's thyroiditis. At 11, she developed psoriasis. At 13, she was diagnosed with alopecia totalis and Epstein-Barr virus (EBV) with resultant and persistent anemia, thrombocytopenia, lymphopenia and neutropenia. She had chronic abdominal pain and diarrhea since age 13. Esophagogastroduodenoscopy revealed lymphocytic esophagitis and active duodenal inflammation with increased intraepithelial lymphocytes. Colonoscopy revealed mildly active chronic colitis with eosinophils. Whole exome sequencing revealed a heterozygous c.239delA (p.Q80Rfs*2) pathogenic mutation in exon 2 of CTLA-4. Family history is remarkable: father (splenomegaly and psoriasis) and brother (autoimmune hemolytic anemia) have CTLA4 haploinsufficiency with the same mutation. Abatacept was initiated with re-growth of hair, improvement in cytopenias, improvement in psoriasis, and some reduction of gastrointestinal symptoms. Since her abdominal pain persisted repeat endoscopies after six months of abatacept revealed persistent active lymphocytic esophagitis with some improvement in inflammatory injury in her duodenum and colon. Physical exam revealed glossitis with a gel-like coating and ulceration on her tongue, xerosis along her face and scalp without other abnormalities (Figure). She denied recent dental procedures, appliances, or tongue biting. Her WBC ranged from 3-4 x10^9 cells/L and hemoglobin 9.4-12.7 g/dL. Absolute lymphocyte count ranged from 1.0- 1.7 x10^9 cells/L. Immunologic evaluation revealed low IgA and pan-low lymphocyte subsets (Table). EBV PCR ranged from 430-1,700 copies/mL. Tongue scraping revealed Candida dubliniensis and she responded to 5 days of fluconazole. Two months later, she developed painful white patches along her tongue and subsequent 4 kilogram weight loss recalcitrant to viscous lidocaine, antacids, and 14 days of fluconazole. Incisional tongue biopsy revealed ulceration with underlying granulation tissue with lymphocyte and plasma cell infiltration consistent with OLP (Figure). Periodic acid-Schiff diastase stain and Grocott stain were negative. Aerobic culture was normal. No fungus was isolated within 14 days. Epstein-Barr encoding region in situ hybridization was negative. Two weeks of topical dexamethasone lead to temporary improvement. Her tongue lesions waxed and waned over the following months. Due to persistent psoriasis, methotrexate was initiated without worsening in her tongue lesion. To our knowledge, this is the first case of OLP reported in a patient with CTLA-4 haploinsufficiency. CTLA-4 haploinsufficiency may present with variable clinical phenotypes including increased risk of EBV viremia and malignancies. Therefore, after EBV and malignancy are ruled out, OLP may be a prudent diagnosis to consider in a CTLA4 insufficient patient with unusual oral lesions.



Informed consent: Informed consent was obtained from all individual participants included in the study.

IgG (751-1,560 mg/dL)	1,170
IgA (82-453 mg/dL)	45
IgM (40-274 mg/dL)	57
IgE (<88 IU/mL)	4
CD3 (1,400-2,200/cumm)	951
CD4 (640-1,200/cumm)	586
CD8 (640-900/cumm)	321
CD19 (260-510/cumm)	124
CD16/56 (180-340/cumm)	28
Vitamin B12 (211-911 pg/mL)	519
Zinc (0.55-1.50 ug/mL)	0.61



(3) Submission ID#756106

An Adult Female With Disseminated Mycobacterium Avium-Intracellulare Found To Have Anti-Interferon-Gamma Autoantibody Syndrome

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Abstract/Case Report Text
Rationale: Anti-interferon-gamma (IFN-γ) autoantibody syndrome is a rare IFN-γ pathway defect presenting with non-tuberculous mycobacterial disease and other opportunistic infections. Onset is usually in the 4th to 6th decade and is likely due to genetic factors. Here we present an adult female with disseminated mycobacterium avium-intracellulare (MAI), found to have high titers of anti-IFN-γ autoantibody.
Methods: An anti-IFN-γ autoantibodies screening assay was performed at the National Institutes of Health.
Results: A 38-year-old female with no past medical history presented with back pain and was found to have bony lytic lesions. She had anemia with hemoglobin 6.2 g/dL, leukocytosis to 25/μL, peripheral eosinophilia to 1100/μL, elevated inflammatory markers, mediastinal lymphadenopathy, and a right sphenoid sinus abnormality. A bronchoscopy and mediastinoscopy were nondiagnostic. A bone marrow biopsy showed decreased trilineage hematopoiesis without evidence of malignancy. Nasal secretion and sinus biopsy showed granulomatous inflammation with MAI. She developed an abscess in her gluteal area at a site of antibiotic administration, and culture grew MAI. An IFN-γ release assay was non-reactive. An anti-IFN-γ autoantibodies screening assay performed at the Laboratory of Clinical Immunology and Microbiology at the National Institutes of Health confirmed the diagnosis of anti-IFN-γ autoantibody syndrome. She was started on a 4-drug regimen for treatment of MAI with significant clinical and radiographic improvement.
Conclusion: A range of molecular defects in the IFN-γ signaling pathway can result in nontuberculous mycobacterial and other opportunistic infections. Anti-IFN-γ autoantibody syndrome is a rare variant that typically presents in adulthood and can be confirmed by an anti-IFN-γ autoantibody assay.

(4) Submission ID#767531

Novel BCL6b Variants Are Associated With Immunodeficiency and Immune Dysregulation

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Abstract/Case Report Text

Introduction: B-cell CLL/lymphoma 6 member B (BCL6b) is a transcriptional repressor which shares close similarity with BCL6, binds to BCL6 DNA binding targets, and interacts with as well as requires BCL6 for its repression activity. The precise role and potential molecular mechanism underlying the involvement of BCL6b in the development of primary immunodeficiency and immune dysregulation is unknown. Herein we report four patients with clinical manifestations of immunodeficiency / immune dysregulation, all found to have rare or novel variants in BCL6b.
Objectives: To describe 4 cases of BCL6b variants and their respective phenotypes.
Methods: The patients were evaluated for possible immune deficiency. A retrospective chart review was conducted examining medical history, diagnosis, laboratory data, and therapeutic responses.

Results: Patient 1 is a 19-year-old Ashkenazi Jewish female who presented at 9 years of age with Neutropenia and later developed immune thrombocytopenia (ITP) and autoimmune hemolytic anemia and cervical lymphadenopathy (biopsy showed reactive changes). Phenotyping showed a high percent of plasmablasts and a low percent of follicular helper T cells, a pattern which could be consistent with abnormal BCL6 function. Treatments included intravenous immunoglobulin (IVIg), rituximab, mycophenolate mofetil (MMF), plaquenil, and belimumab. No infections have been documented. A heterozygous rare variant in BCL6b (c.1348C>T, p.R450W) has been documented.

Patient 2 is a 12-year-old Hispanic male who presented at 5 years of age with chronic abdominal pain, retroperitoneal adenopathy (biopsy with reactive changes), an abdominal mass, pulmonary nodule (biopsy with cytomegalovirus [CMV] inclusions), in association with pancreatitis (biopsy with CMV inclusions) and CMV viremia. Stage IIB Nodular Lymphocyte Predominant Hodgkin's Lymphoma was diagnosed (11 years of age). Intermittent CD4 T cell lymphopenia was documented. Treatments included ganciclovir/valganciclovir (CMV) as well as doxorubicin, bleomycin, vincristine, etoposide, prednisone, and cyclophosphamide (ABVE-PC) for 4 cycles (lymphoma). A heterozygous rare variant in BCL6b (c.1258C>T, p.R420C) has been documented.

Patient 3 is a 10-year-old Middle Eastern female who presented at 7 years with refractory ITP and later developed Evan's syndrome and splenomegaly. Immunophenotyping showed B-cell lymphocytosis, low memory CD4 T cells and low NK cells. Treatments included systemic steroids, IVIg, MMF and romiplostim. A homozygous rare BCL6b variant (c.118C>T; p.T373M) was identified.

Patient 4 is a 47-year-old Caucasian female who presented at 40 years of age with recurrent fever, abdominal pain, liver function abnormalities and persistent Epstein-Barr virus (EBV) viremia. Biopsies showed Epstein-Barr encoding region positive (EBER+) T cells in the liver, bone marrow and spleen (underwent splenectomy). Naïve CD4 T cell lymphopenia was documented. Treatments included solumedrol and rituximab. Persistent EBV viremia was the sole infectious issue noted. A heterozygous rare variant in BCL6b (c.793T>G, p.F265V) has been documented.

Conclusions:

Mutations of BCL6b appear to be associated with lymphoproliferation and autoimmunity as well as susceptibility to Herpes virus infections. Additional research focusing on characterization of DNA binding sites of BCL6b as well as the downstream expression of associated target genes is needed. These data combined with longitudinal analysis of additional patients with confirmed BCL6b mutations, will help clarify determinants of BCL6b pathogenesis and highlight potential therapeutic strategies.

	Patient	1	2	3	4
	Age	20y/o female (Ashkenazi Jew)	12y/o male (Hispanic)	10y/o female (Middle Eastern)	47 female (Caucasian)
Variant	**Mutation**	Heterozygous c.1348C>T, p.R450W, 4th Zinc finger	Heterozygousc.1258C>T, p.R420C, 5th Zinc finger	Homozygous c.118C>T; p. T373M, 2nd Zinc finger	Heterozygous c.793T>G, p.F265V
	Gnomad AF	0.00003188	0.00006416	0.00002406	0.000008015
	Polyphen; SIFT	Probably damaging; Deleterious	Probably damaging; Deleterious	Probably damaging; Deleterious	Benign; tolerated
Onset	Age at onset	9y/o	5y/o	7y/o	40y/o
	Presenting symptoms	Weakness, neutropenia, Hashimoto's	Chronic abdominal pain and retroperitoneal mass. Lung lesion. Bx - pancreatitis and CMV inclusion bodies + CMV pneumonitis.	Refractory ITP	Recurrent fever, abdominal pain, LFT abnormalities
Immune Dysregulation	Hematologic	Neutropenia at 9; ITP at 15.5; AIHA at 16.5	At 5yr - Recurrent abdominal pain with extensive retroperitoneal, periportal and peripancreatic LAD. At 8yr - Worsening LAD + splenomegaly. Mesenteric LN Bx - paracortical hyperplasia and atypical T cell infiltration. At 11yr - Mediastinal, hilar, and axillary adenopathy. LN biopsy showed lymphocyte predominant Hodgkin lymphoma.	Evan's syndrome, ITP, DAT+ AIHA and pancytopenia, resolved with immunosuppression. Splenomegaly.	Splenomegaly. Splenectomy showing granulomas.
	Skin	Alopecia areata - on and off between 9-15. More recent psoriatic rash (per Bx).			
	GI	Rec aphthous stomatitis		Intermittent vomiting and diarrhea	
	Urinary		Age 8yrs - Edema, proteinuria and AKI (Cr-1.6). Bx - Acute tubule-interstitial nephritis		
	Endocrine / other	Hashimoto's thyroiditis at 9			3 mm nodules in lung bases
Infections	Viral	None	CMV viremia at presentation (6000 copies). Responded to ganciclovir / valganciclovir.	None	Persistent EBV viremia
Immune Evaluation	Lymphocyte Subsets	Normal	Fluctuating lymphopenia (mostly CD4 lymphopenia).	B cell lymphocytosis (24%, 694/uL),	
	B cell phenotyping	Low CD38lowCD24high memory B cells. High % of plasmablast. Increased BAFF-R MFI,		High % of CD38high cells (plasmablasts?)	Post rituximab – No B cells

(continued)

		increased CD27 MFI, Increased % of class-switched memory cells.			
	T cell phenotyping	CD4s - Skewing toward CD45RO+CCR7- Teff. CD8s - absent CD45RO+ CCR7+ Tcm. High % of CD45RA+CCR7- Temra cells.		Low CD56+ cells, Low CD45RO+ memory CD4+ T cells.	Reduced naïve CD4 cells
	DNT	Normal - 0.97% TCR⊡⊡+ DNT		Elevated – 7.6% TCR⊡⊡+ DNT	normal
	Antibody levels	2015-2018: Elevated IgG between 1507 to 2079mg/dL. Normal IgM.	Normal IgG. Low IgM in the past.	Normal - IgA 98, IgG 1007, IgM 146	Low IgG and Low IgM, normal IgA (post rituximab)
	Autoantibodies	Positive APLA at 12 (resolved)		Positive ANA, anti-dsDNA and anticardiolipin (anti-dsDNA resolved)	
	Other	Normal Treg phenotyping	Normal mitogen proliferation. Suboptimal vaccine response.	normal mitogen and vaccine titers, normal NK function	
Pathology	BM Bx	Normal - 2016	Normal - 2013, 2016	2015 - Megakaryocytic number and maturation compatible with peripheral destruction	Increased EBV+ T cells and granulomas
	LN Bx	Reactive - 2016	2015 - Negative for malignancy. 2016 - paracortical hyperplasia + atypical T cell infiltration. 2018 - HD.		
	Other	Skin - Early lesion of psoriasis - 2016	2016 - Kidney Bx - Acute tubule-interstitial nephritis		Splenectomy - necrotizing granulomas, EBV+ T cells., Liver biopsy - EBV hepatitis with EBV in T cells, erythrophagocytosis

(5) Submission ID#769441

Unexpected Autoimmunity and Infections in A Cohort of Patients with Complement Deficiencies

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Abstract/Case Report Text
Introduction: Patients with complement deficiencies represent globally less than 2% of all primary immunodeficiencies (PID). Sometimes, diagnosis is not straighforward and diagnostic delay may cause severe sequelae. We report a case series of 11 patients with complement deficiencies. Aim: To describe the epidemiology of patients with complement deficiencies in a single Institution in Spain, clinical characteristics prior to diagnosis, site of infection and microorganisms involved as well as treatment procedures and outcomes.

Methods: Retrospective study. Review of clinical charts of patients diagnosed with complement deficiency (N= 11).
Results: Male 36%. Median age at diagnosis 44 months (+/-SD 37). The distribution by defect is: C2 (63%), FI (27%), FH (10%). Infections prior to diagnosis were: Pneumoniae (45.5%, mean 1.5 episodes), meningitis (36%), AOM (36%, mean 1), bacteremia (36%), cellulitis (27%), mastoiditis (18%), septic arthritis (18%).The microorganisms isolated were: S. pneumoniae (70%), N. meningitidis (10%), H. influenzae (10%) and N. fowleri (10%). The patient with complement factor 2 deficiency and N. fowleri infection is almost intact. In adition, one patient developed chronic lung disease (bronchiectasis) prior to diagnosis. All patients were vaccinated with antimeningococcal tetravalent conjugated,MenB, PCV13 and Pneumovax. In adition, all patients were under antibacterial prophylaxis. Concerning autoimmune manifestations, 2 out of 11 patients, both of them with complement Factor I deficiency (18%) had recurrent Henoch-Schönlein purpura (HSP). The reasons for immunological work-up were: recurrent respiratory infections (44%), affected sibbling (18%), HSP (18%), meningitis (10%), atypical site of infection and/or microorganism (18%). Currently, all of them are alive and well since diagnosis without any breakthrough infections.
Conclusion: Not only recurrent infections may lead to diagnosis in PID patients with complement deficiencies, but also autoimmune manifestations. Despite the fact that in our population the vast majority of isolations were typical well-known capsulated microorganisms, we show that Naegleria fowleri infection should prompt assessment for complement deficiency. As our patient has survived to a primary amebic meningoencephalitis (PAM) we can speculate on the role of C2 in PAM survival. However, further studies are needed to unravel the underlying mechanism.

(6) Submission ID#769449

Identification of Novel NLRC4 And Il2RA Variants In A Family Cohort With Juvenile-Onset Arthritis And Rash

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Abstract/Case Report Text

Introduction: Identification of genetic etiologies of autoinflammatory syndromes can inform targeted therapy to improve outcomes.

Objectives: We aimed to identify a genetic etiology for an underlying autoinflammatory syndrome in a 3-year-old boy presenting with failure to thrive, rash, and polyarthritis since infancy without significant fevers. The patient's mother and maternal aunt also had similar symptoms since infancy.

Methods: We obtained a history, exam, routine laboratory evaluation, chromosomal microarray, immune phenotyping and functional assays, and genetic sequencing of familial autoinflammatory syndromes via INVITAE.

Results: The patient's first examination showed small and large joint polyarthritis and maculopapular rash. Laboratory results included anemia, positive ANA, negative RF and anti-CCP, mildly raised ferritin, and very elevated platelet level, LDH, ESR, CRP and IgG. He had recurrent diarrhea and tested positive for Campylobacter. INVITAE's autoinflammatory panel showed two heterozygous variants of unknown significance (VUS): NLRC4, exon 4, c.741_742insAlu (p.Leu247fs) and IL2RA, exon 2, c.76G>C (p.Asp26His).

Genetic testing revealed the same two variants in the patient's mother and aunt, while unaffected relatives had one or the other (see Figure 1). The patient and mother are HLA-B27+ and have the same 2.8 Mb duplication from 3q28 to 3q29 on chromosomal microarray including IL1RAP. Signal transducer and activator of transcription phosphorylation (pSTAT5) studies showed increased baseline pSTAT5 induction without IL-2 stimulation in patient compared to control.

The patient's arthritis partially improved with naproxen and steroid joint injections. Given genetic results, subcutaneous anakinra was initiated at 10 mg/kg daily with significant improvement in arthritis, rash, and fatigue. Labs after one month showed resolved anemia, normal inflammatory markers, and high IL-18 (7,824 pg/mL, normal 89-540). He switched to 4.29 mg/kg of canakinumab monthly. After one month, he had mildly active arthritis, occasional fatigue, and stable labs apart from an increase of IL-18 to 15,329 pg/mL. The mother, who is poorly controlled off therapy, also has elevated IL-18 (7,176 pg/mL). See Figures 2-4 for physical exam findings and Table 1 for a summary of symptoms and results.

Conclusion: We present a family cohort with juvenile-onset arthritis and rash, found to have elevated IL-18, VUS in both NLRC4 and IL2RA, and a chromosomal duplication consistent with a heritable autoinflammatory syndrome. While it appears that both variants are required for symptomatology, his presentation is most consistent with an unrecognized gain of function NLRC4 mutation despite lack of recurrent fevers or enterocolitis. Genetic evaluation led to targeted therapy and improved outcomes, with additional testing underway to further personalize therapy.

The patient's family consented for publication.

References:

1. Canna SW, et al. An activating NLRC4 inflammasome mutation causes autoinflammation with recurrent macrophage activation syndrome. Nature Genetics. 2014;46(10):1140-6.

2. Romberg N, et al. Mutation of NLRC4 causes a syndrome of enterocolitis and autoinflammation. Nature Genetics. 2014;46(10):1135-9.

- Patients' (**III.1**) INVITAE autoinflammatory panel revealed two heterozygous variants of unknown significance (VUS):
  - ***NLRC4*, exon 4, c.741_742insAlu (p.Leu247fs)**
  - ***IL2RA*, exon 2, c.76G>C (p.Asp26His)**
- **III.1 and II.3** have the same 2.8 Mb duplication from 3q28 to 3q29, which includes IL1RAP. This is not present in II.2.

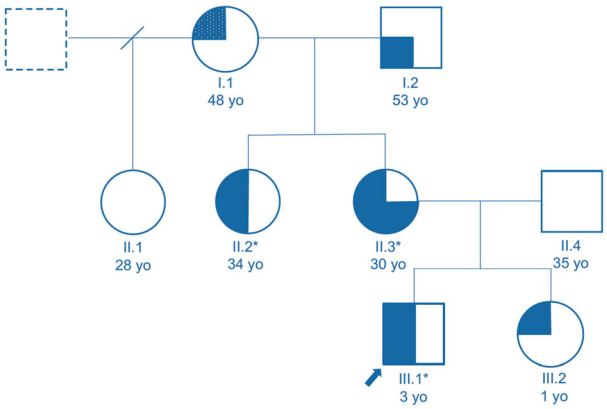

Figure 1, INVITAE Autoinflammatory Syndrome Panel Results.

Table 1, Symptoms and Lab Results for Affected Individuals.

		PATIENT (III.1)				MOTHER (II.3)	AUNT (II.2)
		Initial Labs (no therapy)	After 1 month of 10 mg/kg subQ Anakinra daily	1 month after switch to 4.29 mg/kg subQ Canakinumab	After 4 doses of monthly Canakinumab	Off Therapy	Off Therapy
Signs and Symptoms	Normal Ranges	Small/large joint polyarthritis, maculopapular rash, failure to thrive, fatigue, decreased appetite, diarrhea. No fevers.	Significantly improved arthritis, fatigue, and appetite. Resolution of rash.	Mildly active arthritis, mild fatigue, new rash on legs.	Active arthritis, new rash still present.	- Small/large joint erosive polyarthritis, tenosynovitis, maculopapular rash, psoriasiform rash, short stature, fatigue. - Failed TNF inhibitors.	- Small/large joint polyarthritis, maculopapular rash, short stature. - Childhood: failure to thrive, ESR 97, anemia, delayed bone age, maculopapular rash.
WBC	5.7 - 10.5	9.4	7.67	5.62	6.18	6	4.69
Hgb	10.3 - 13.8	7.2	11.3	11.7	10.8	13.4	12.6
Platelets	150 - 500	1,090	555	464	590	376	242
ESR	0 - 15	> 130	20	33	36	29	18
CRP	< 1	17.2	1.2	2.7	3.2	2.4	1.9
LDH	500 - 920	1,100	987	1,758	908	1,015	1,959
Ferritin	10 - 60	108	14.6	24.1	13	41	51.5
IgG	413 - 1112	1,884	1,176	1,257	1,239	1,410	
IL-18	89 - 540		7,824	15,329		7,176	15,094
Serologies		+ANA 1:320 speckled, +HLA-B27, -RF, -Anti-CCP				-ANA, +HLA-B27, -RF, -Anti-CCP	+ANA1:160, speckled
Other		Normal Tregs, urinalyses, transaminases, creatinine, +Campylobacter in stool, abnormal pSTAT5*				Normal Tregs, pSTAT5, and CD4+/CD8+ cells	sIL-2R 334

*pSTAT5 studies showed increased baseline pSTAT5 induction without IL-2 stimulation in patient compared to control. Repeated with same results.

Figure 2, Arthritis in Mother's Hand. Age 30, off therapy.

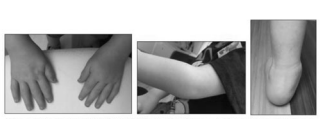

Figure 3, Persistent Arthritis in Patient after 4 Doses of Monthly Canakinumab. Age 3 years 10 months.

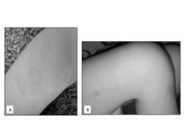

Figure 4, Cutaneous Manifestations in Patient. A: Migratory erythematous lesions since infancy that resolved with anti-IL-1 therapy. B: New rash since starting Canakinumab.

Informed consent: Informed consent was obtained from all individual participants included in the study.

(7) Submission ID#769608

Age-Related Transcriptional Modules and TF-miRNA-mRNA Interactions in Neonatal and Infant Human Thymus

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Abstract/Case Report Text
Background – The human thymus suffers a transient neonatal involution, recovers and then starts a process of decline between the 1st and 2nd years of life. Age-related morphological changes in thymus were extensively investigated, but the genomic mechanisms underlying this process remain largely unknown.
Methods – Through WGCNA and TF-miRNA-mRNA integrative analysis we studied the transcriptome of neonate and infant thymic tissues grouped by age: 0-30 days (A); 31days-6 months (B); 7-12 months (C); 13-18 months (D); 19-31months (E). Age-related transcriptional modules, hubs and high gene significance (HGS) genes were identified, as well as TF-miRNA-hub/HGS co-expression correlations.
Results – Three transcriptional modules were correlated with A and/or E groups. Hubs were mostly related to cellular/metabolic processes; few were differentially expressed (DE) or related to T-cell development. Inversely, HGS genes in groups A and E were mostly DE. In A (neonate) one third of the hyper-expressed HGS genes were related to T-cell development, against one-twentieth in E, what may correlate with the early neonatal depletion and recovery of thymic T-cell populations.
Conclusions – Age-related thymic processes are tightly regulated by TF-miRNA-hub/HGS interactions that govern differentially cellular and molecular processes involved in the functioning of the neonate thymus and in the beginning of thymic decline.
FAPESP 2014/50489-9

(8) Submission ID#773934

A Pilot Study on the Use of Diagnostic Exome Sequencing in Premarital Screening for Primary Immunodeficiency

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Abstract/Case Report Text
Consanguineous marriages in tribal cultures, such as that in the United Arab Emirates significantly increase the prevalence of autosomal recessive disorders. Premarital genetic screening and counseling, thus, are expected to reduce the frequency of these diseases.
In this pilot study, diagnostic exome sequencing was used in the premarital screening program to identify recessive pathologic variants preventable by premarital counseling. A total of 487 pathologic or likely pathologic variants were identified in 176 studied Emiratis (88 couples), averaging 2.8 variants per person. Four percent of the persons had negative diagnostic exome sequencing; the remaining had one to eight variants per person. Of the 351 distinct variants, 162 (46%) were novel. Twenty (23%) couples had pathologic or likely pathologic variants of inborn errors of immunity (IEI). Two couples (10%) had IEI pathologic or likely pathologic heterozygous variants in both partners imposing risk for autosomal recessive disease in the offspring. Other eighteen couples (90%) had pathologic/likely pathologic heterozygous variants present in only one person of the couple. Total of sixteen (4.5%) IEI variant identified and eight (50%) were novel. Fourteen known phenotypic IEI diseases were recognized (table. 1). These preliminary results support a need for nationwide premarital genetic screening, and primary immunodeficiency registry to identify common and novel pathogenic variants with high heritability rate. These results will aid adopting a pre- and post-connectional reproductive carrier counseling to reduce autosomal recessive diseases. Also, it will assist the diagnosis of these complex diseases in our community.

Table 1 Pathologic or likely pathologic variants of primary immunodeficiency (PID).

Diseases (MIM)	*Genes (MIM)*	*Variants*
IMD20 (615707)	*FCGR3A* (146740)	**NM_001127593.1:c.423dupT (p.Ile142Tyrfs)**
HIES2 (243700)	*DOCK8* (611432)	**NM_203447.3:c.5287C>T (p.Arg1763fs)**
CD8DF (608957)	*CD8A* (186910)	**NM_001145873.1:c.49+2T>G**
C7D (610102)	*C7* (217070)	NM_000587.3:c.1135G>C (p.Gly379Arg) - VCV000012108
		NM_000587.3:c.405delT (p.Asn136Thrfs) - VCV000432759
ADA-SCID (102700)	*ADA* (608958)	NM_000022.3:c.226C>T (p.Arg76Trp) - VCV000001962
		NM_000022.3:c.454C>A (p.Leu152Met) - VCV000001979
IMD28 (614889)	*IFNGR2* (147569)	**NM_005534.3:c.123C>G (p.Tyr41Ter)**
BLS-I (604571)	*TAP2* (170261)	**NM_000544.3:c.753dupA (p.Arg252Thrfs)**
GS2 (607624)	*RAB27A* (603868)	**NM_183235.2:c.514_518delCAAGC (p.Gln172Asnfs)**
ISDNA (617425)	*EXTL3* (605744)	NM_001440.3:c.1970A>G (p.Asn657Ser) - VCV000417795
CFDD (613912)	*CFD* (134350)	**NM_001928.2:c.285C>A (p.Tyr95fs)**
Ficolin 3 deficiency (613860)	*FCN3* (604973)	NM_003665.3:c.349del (p.Leu117Serfs) - VCV000005285.1
CDG1 (233700)	*NCF1* (608512)	NM_000265.5:c.579G>A (p.Trp193Ter) - VCV000426990
FI SCN7 (617014)	*CSF3R* (138971)	**NM_156039.3:c.1015delG (p.Asp339Thrfs)**
NBSLD (613078)	*RAD50* (604040)	NM_005732.3:c.2165dupA (p.Glu723Glyfs) - VCV000141045

Bolded variants were not found at https://www.ncbi.nlm.nih.gov/clinvar/ or HGMD® Professional 2019.2; MIM, Mendelian Inheritance in Man; VCV, Variation in ClinVar; rs, Reference SNP; IMD20, immunodeficiency 20; *FCGR3A*, Fc fragment of IgG, low affinity IIIa, receptor for; HIES2, hyper-IgE

recurrent infection syndrome 2, autosomal recessive; *DOCK8*, dedicator of cytokinesis 8; CD8DF, CD8 deficiency, familial; *CD8A*, CD8 antigen, alpha polypeptide; C7D, complement component 7 deficiency; *C7*, complement component 7; ADA-SCID, severe combined immunodeficiency, autosomal recessive, T cell-negative, B cell-negative, NK cell-negative, due to adenosine deaminase deficiency; *ADA*, adenosine deaminase; IMD28, immunodeficiency 28; *IFNGR2*, interferon-gamma receptor 2; BLS-I, bare lymphocyte syndrome type I, *TAP2*, transporter, ATP-binding cassette, major histocompatibility complex, 2; GS2, Griscelli Syndrome, Type 2; *RAB27A*, RAS-associated protein RAB27A; ISDNA, immunoskeletal dysplasia with neurodevelopmental abnormalities; *EXTL3*, exostosin-like glycosyltransferase 3; CFDD, complement factor D deficiency; *CFD*, complement factor D; *FCN3*, ficolin 3; CDG1, granulomatous disease, chronic, autosomal recessive, cytochrome b-positive, type I; *NCF1*, neutrophil cytosolic factor 1; SCN7, neutropenia, severe congenital, 7, autosomal recessive; *CSF3R*, colony-stimulating factor 3 receptor, granulocyte; NBSLD, Nijmegen breakage syndrome-like disorder; *RAD50*, S. cerevisiae, homolog of; CDG1H, congenital disorder of glycosylation, type Ih; *ALG8*, ALG8, S. cerevisiae, homolog of.

10) Submission ID#778505

Malignancy in Common Variable Immune Deficiency: Data from the IDEaL Patient Registry

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Abstract/Case Report Text

Introduction: Common variable immune deficiency (CVID) is associated with an increased risk for development of several types of malignancies including lymphoid and gastrointestinal cancers. With improvement in overall survival and longevity in this patient population largely due to the effectiveness of immunoglobulin replacement therapy (IGRT) in reducing infections, cancer has now emerged as the one of the most significant life-threatening complications of CVID.

Objective: The purpose of this study was to evaluate the prevalence of cancers in patients with CVID receiving IGRT in the home.

Methods: Data were analyzed from patients with a diagnosis of CVID (ICD-10 codes: D83.9, D83.1) that were enrolled in the IDEaL (Immunoglobulin, Diagnosis, Evaluation, and key Learnings) Patient Registry between 2010 and 2019. This is a prospective, longitudinal registry study of patients receiving IGRT in the home or ambulatory infusion suite with one national home infusion provider. Cancer occurrence and type, patient demographics and IGRT dosing information were obtained from medical charts and nursing and pharmacy standard of care forms.

Results: Out of 312 patients with CVID, 38 (12.2%) patients developed 41 cancers. The incidence of hematological malignancies was 6.1% (19 cases) and accounted for 46.3% of all reported cancers. There were 11 (26.8%; 11/41) cases of Non-Hodgkin's Lymphoma (NHL), 7 (17.1%; 7/41) cases of Chronic Lymphocytic Leukemia (CLL), and 1 (2.4%; 1/41) case of multiple myeloma. Of the cases of NHL, 2 cases were identified as gastric mucosa-associated lymphoid tissue (MALT) lymphoma. The incidence of solid tumors was 7.1% (22 cases). These accounted for 53.7% of all reported cancers and were heterogeneous in localization. There were 7 (17.1%; 7/41) cases of skin tumors (3 melanomas, 2 basal cell carcinomas, and 2 in which cell type was not-specified). There were 4 (9.8%; 4/41) cases of breast cancer, 3 (7.3%; 3/41) cases of lung cancer, 2 (4.9; 2/41) cases of uterine cancer, 2 (4.9% 2/41) cases of prostate cancer, 2 pituitary tumors (4.9%; 2/41), 1 (2.4%; 1/41) bladder tumor, and 1 (2.4%; 1/41) adrenal carcinoma. Patients who developed cancer were mostly female: 24 (63.1%), and the median age at cancer diagnosis was 63 (range 21-75 years). The prevalence of all cancers in this Registry population was higher than the 5-year, all age, all cancers limited duration prevalence estimates in the US (1.5%). The median age at time of referral for home infusion of IGRT was 65 years of age. Of these patients, 55.3% were Ig naïve prior to starting service with this home infusion company. Most patients received subcutaneous Ig (71.1%) versus intravenous Ig (28.9%) at start of care with mean doses of 128.6±42.4 mg/kg/wk and 431.0±70 mg/kg/month respectively.

Conclusions: Over 12% of the study population suffered from a malignancy. These data point to defective immunosurveillance mechanisms in preventing certain cancers in patients with CVID. Better understanding the occurrence of malignancy in patients with CVID will help to postulate mechanisms between immune factors and cancer initiation and progression as well as appropriate screening and treatment patterns for this patient population including the role of IGRT.

(12) Submission ID#781367

Multiple Dermatofibrosarcoma Protuberans: a Phenotype Unique in ADA deficient SCID

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Abstract/Case Report Text

DFSP is a rare low-grade cutaneous malignancy. A slowly progressive tumor that rarely metastasizes but frequently has broad subclinical extension and a high recurrence rate after excision. DFSP rare in children, but is very common in ADA-deficient SCID and all patients should have full dermatology screening at least annually. It was first identified in the ADA population in 2011 and is not seen in X- SCID or other PID. Protuberant lesions should be referred for Mohs micrographic surgery.

(13) Submission ID#781460

Rare Presentation Of Ataxia-Telangiectasia On Newborn Screen

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Abstract/Case Report Text

Introduction: Ataxia-telangiectasia (AT) is a rare disease of primary immunodeficiency and has a wide spectrum of immunologic abnormalities. We present a case of a patient born with low T-cell receptor excision circles (TRECs) with progressive combined immunodeficiency leading to the diagnosis of T-B-NK-SCID and treatment with bone marrow transplant, who later presented with L-dopa responsive dystonia and finally was diagnosed with AT after genetic testing was done in his grandmother that had breast cancer.

Case presentation: A full term male was born with low TRECs (44 copies/uL) on newborn screen within the first few months of screening in Illinois. He presented to clinic at 2 months of age, where he was found to have hypogammaglobulinemia and a predominant B cell deficiency with mildly reduced T cells. He was started on monthly IVIg and prophylactic antifungals while initiating his evaluation. B cell tyrosine kinase (BTK) was normal and T/B cell mitogen stimulation testing was normal. However, he had a steady decline in T and NK cell counts over months. Genetic testing that was available at that time included ADA, AK2, DCLRE1C, LIG4, NHEJ1, RAC2, RAG1, RAG2 which only revealed heterozygosity for the V186L variant of the DCLRE1C gene, which was also carried by his unaffected father and sister. Due to the persistent decline his CD3 counts to 147/uL and CD56/16 counts to 26/uL, at age 10 months he received a matched sibling bone marrow transplant conditioned with campath. He achieved complete immune reconstitution by 18 months after the transplant. He had normal development until age 2 when he presented with occasional falling after walking normally and was diagnosed with L-dopa responsive dystonia. His grandmother developed breast cancer, and because of a strong family history of breast cancer had genetic screening that revealed a mutation in ATM. At this point the patient was evaluated for AT and found to also be affected.

Discussion: The immunodeficiency in AT can have a wide variety of presentations involving humoral or cell-mediated deficiency or occasionally both. AT is known to present with increased NK cells and the immune defects are typically non-progressive in nature. This patient however had progressive immune deficiency necessitating transplant as well as reduction in NK cells. AT presenting as T-B-NK-SCID is atypical.

Conclusion: The immunologic defects in AT are exceedingly variable. This case suggests that AT may present with a picture similar to T-B-NK-SCID. Luckily, the majority of genetic screening programs have now incorporated ATM into their panels, so most patients will be captured, as opposed to early on in the era of TREC newborn screening. This case also highlights the importance of early consideration of AT in patients born with low TRECs, as syndromic manifestations, including dystonia, may present later in life.

(14) Submission ID#784536

Chronic Granulomatous Disease With Inflammatory Bowel Disease: Disease Presentation, Treatment, And Outcomes From The USIDNET Registry

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Abstract/Case Report Text

Background: Chronic granulomatous disease (CGD) is a primary immunodeficiency disorder caused by defects in the phagocytic NADPH oxidase complex, leading to increased susceptibility to infection and inflammatory or autoimmune disease. Up to 50% of patients have gastrointestinal (GI) involvement and meet diagnostic criteria for inflammatory bowel disease (CGD-IBD).

Objectives: We analyzed CGD patients from the United States Immunodeficiency Network (USIDNET) registry to determine whether IBD may change the presentation, treatment, and outcomes of CGD patients, as compared to those without IBD.

Methods: A retrospective evaluation of CGD cases from the USIDNET registry was completed. CGD-IBD was defined as the presence of any major physician-reported inflammatory, non-infectious GI tract disease manifestation, including Crohn disease, ulcerative colitis, IBD endoscopy findings, GI fistulas, GI strictures, GI obstruction, and proctitis. Demographic information, genotypes, symptoms and conditions, infections, antimicrobial therapies, immunomodulator use, and allogeneic hematopoietic stem cell transplantation (HSCT) data were analyzed.

Results: 194 patients with a diagnosis of CGD were identified. 96 met criteria for IBD; 98 were categorized in the non-IBD group. Crohn disease and colitis were the most common GI disease manifestations in the CGD-IBD group (n=79), followed by GI fistulas (n=22). CGD-IBD patients had an increased average frequency of infections (10.6 events/patient) compared to the CGD-non-IBD group (5.1 events/patient). In both groups, lower respiratory tract infections were the most common infection type and Aspergillus was the most common organism. Enteric organism infections were more common in IBD patients. Temporal data regarding the timing of infections were not available. Immunomodulators, including biologics and interferon-gamma, were used at a significantly higher rate in IBD patients compared to non-IBD patients (80% versus 55%, $p = 0.0003$). The presence of IBD, as compared to no IBD, in a CGD patient increased the odds of immunomodulator use (OR = 3.168) (95% confidence limit 1.669-6.017, $p = 0.0004$). Patients who received immunomodulator treatment had a higher average number of infections as compared to those who did not. Thirty-one percent of all patients underwent HSCT; 8 patients died after undergoing HSCT, of which 4 had CGD-IBD. Of the entire CGD cohort, 17 patients died (8.8%), with a median age of death of 21.8 years (range 2.5-48.1 years), and with no significant difference between IBD and non-IBD patients (p=1.00).

Conclusions: Infectious events, enteric organism infections, and immunomodulator use were higher in IBD than non-IBD patients, though mortality was not increased. Patients with CGD and concurrent IBD are at increased risk for complications, supporting the importance of early recognition and diagnosis. Due to limitations in the available data, we are unable to conclude whether the increased number of infections in CGD-IBD patients was due to presence of IBD, or other factors such as age, use of immunomodulators, or transplant status. The USIDNET data may reflect more effective antistaphylococcal prophylaxis and treatment in the current era, as compared to historical data. Our findings reinforce the frequent coexistence of IBD and CGD, illustrate major phenotypic features of CGD patients with GI inflammatory conditions, and highlight the need for further investigations.

(15) Submission ID#785311

Three Copies of Four Interferon Receptor Genes Underlie Type I Interferonopathies In Down Syndrome

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Abstract/Case Report Text
Down syndrome (DS) is characterized by the occurrence of three copies of human chromosome 21 (HSA21). These patients often develop chronic mucocutaneous candidiasis (CMC) and autoimmune thyroiditis, mimicking patients with heterozygous gain-of-function (GOF) STAT1 mutations, which enhance cellular responses to the three types of interferon (IFN). HSA21 contains a cluster of four interferon receptor (IFN-R) genes: IFNAR1, IFNAR2, IFNGR2 and IL10RB. A gene dosage effect at these four loci may contribute to the infectious and autoimmune manifestations observed in individuals with DS. We report high levels of IFN-αR1, IFN-αR2 and IFN-γR2 expression on the surface of monocytes and EBV-transformed-B (EBV-B) cells from DS patients. Levels of IFN-γR1, encoded by a gene on chromosome 6, were similar in the immune cells of DS patients and healthy controls. Total and phosphorylated STAT1 (STAT1 and pSTAT1) levels were constitutively high in unstimulated and IFN-α- and IFN-γ-stimulated monocytes from DS patients, although less so than those in patients with GOF STAT1 mutations. Following stimulation with IFN-α or -γ, but not with IL-6 or IL-21, pSTAT1 and IFN-γ activation factor (GAF) DNA binding activities were significantly higher in the EBV-B cells of DS patients than in controls, this response resembling the dysregulated responses observed in patients with STAT1 GOF mutations. Plasma type I IFNs concentrations were high in about 12% of the DS patients tested. A genome-wide transcriptomic analysis involving principle component analysis and a comparison of interferon modules was performed on circulating monocytes. It showed that IFN-stimulated genes (ISGs) were expressed more strongly in DS than in controls. DS monocytes have intermediate levels of IFN-α- and IFN-γ- induced ISGs relative to monocytes from healthy controls and from patients with GOF STAT1 mutations. By contrast to patients with GOF STAT1 mutations, circulating Th17 counts were normal and the proportion of terminally differentiated CD8+ T cells was high in DS patients. The constitutive upregulation of type I and type II IFN-R, at least in monocytes of DS patients, may therefore contribute to the autoimmune diseases observed in these individuals.

(16) Submission ID#785796

Gotta Be More Than Just Warts, GATA 2 Deficiency

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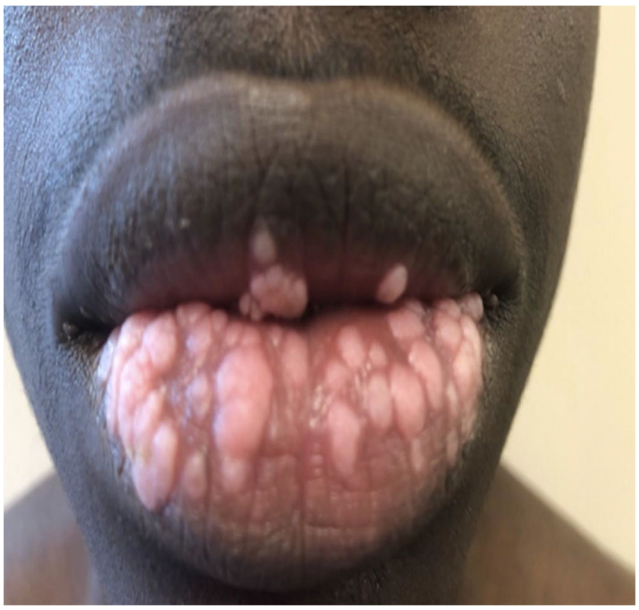
Abstract/Case Report Text
Background: Warts are benign growths produced by human papilloma virus (HPV) that largely affect immunocompetent children and adolescences. Warts are common in the pediatric population due to their naïve immune system that has not yet mounted a protective response to HPV.

Usually by their teenage years most are immune to the virus and warts have regressed. Though mechanism isn't exactly known, evidence has shown cellular immunity specifically T-cells and Natural Killer (NK) cell cytotoxic response is essential for wart regression. Hence, patients with abundant, recurrent, and unmanageable warts should warrant further testing for possible underlying immune defect.

Case Presentation: 16-year-old Haitian male with 8-year history of multiple verrucous lesions on the oral mucosa and lips unresponsive to medical treatments (Acyclovir, Aldara and cimetidine) and laser therapy. He had no recurrent pneumonias, sinusitis, otitis media, meningitis, sepsis, deep-seated infections, abscess, recurrent or intermittent fevers, stomatitis, or autoimmunity. His mother had died of malignancy and is father and half siblings (paternal side) were alive and well. Physical exam revealed several verrucous lesions covering the entire lower lip and part of the upper lip, the tongue and soft palate. Smaller verrucous lesions noted on the fingers. No genital or anal warts. There was a dry, non-pruritic, mildly scaly, faint rash on the back and chest.

Oral lesions were biopsied and pathology showed epithelial hyperplasia consistent with HPV. Immune evaluation revealed the following results: CBC with diff relevant for leukopenia, neutropenia, lymphopenia and monocytopenia. Serum immunoglobulins were within normal. Lymphocyte subset showed B, NK, CD3+ and CD4+ lymphocytopenia. Lymphocyte antigen and mitogen proliferation studies significant for low PWM, ConA, Candida stimulation. He also had low functional antibodies to pneumococcal vaccine (4/23 protective). GATA2 deficiency was strongly suspected due to the presence of recurrent and intractable HPV + warts along with severe circulating monocytopenia, B-cell, NK-cell and CD4 lymphocytopenia. Genetic testing revealed a GATA2 heterozygous pathogenic variant c.1114G>A due to a missense mutation. Upon his diagnosis, NIH was contacted and he was enrolled in Dr. Steven Holland's GATA-2 Clinical Trial.

Conclusions: We report a teenage Haitian boy with indolent HPV + warts and profound monocytopenia, B-cell, NK-cell and CD4-cell lymphocytopenia found to have a pathogenic variant on GATA2 exon 5, which reportedly has only been previously observed in five other individuals affected with GATA2 deficiency, all presenting at different age of onset with a wide spectrum of manifestations, phenotypes and outcomes. This demonstrates that even patient's with same GATA2 mutation do not present with distinguishing features for a particular pathogenic variant. Our patient's unique manifestation is just another step closer in unraveling the complex pathogenesis and diverse phenotypes found in GATA2 deficiency.



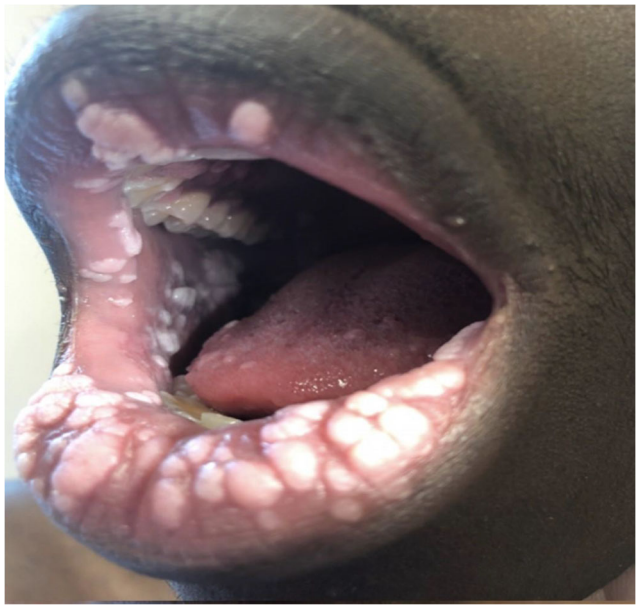


Table-1: Comparison of Patient's with same GATA2 Missense Mutation (c.1114G>A) and Diverse Manifestations

Patient	Sex	GATA2 Mutation	Overall Phenotype	Age of Initial Manifestation (yr)	Infections	Other features
1	F	c.1114G>A	Neutropenia, Lymphopenia, Monocytopenia, Anemia	At 13 with AML	Pulmonary aspergillus, Pneumonia, Skin lesions, recurrent oral HSV, GI infections	Died at 18 from H1N1 infection
2	F	c.1114G>A	B & NK Lymphopenia, MDS	At 66 with MDS	none reported	
3	F	c.1114G>A	MDS	At 42 with MDS	Esophageal candidiasis, pneumonia	Dermo-hypodermitis, arthritis, Lymphedema, Breast Cancer, DVT, Died at 72
4	unk	c.1114G>A	AML	At 20 with AML	Pneumonia EBV-related	Died at 21
5	unk	c.1114G>A	none reported	22	HPV infection	Pulmonary involvement
6	M	c.1114G>A	B, NK and CD4 Lymphocytopenia Monocytopenia	At 16 with HPV infection	Multiple oral warts HPV	

Informed consent was obtained from all individual participants included in the study.

(17) Submission ID#786488

Humoral and Cell Mediated Immune Dysregulation Identified by Newborn Screening

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Abstract/Case Report Text

Introduction Screening for severe combined immunodeficiency (SCID) has been widely implemented as a part of the newborn screen. Testing is done by measuring T cell receptor excision circles (TRECs) which are DNA excision fragments that can be used as an indirect measure of naïve T cell production. The goal of this project was to evaluate all immunologic conditions identified in infants who have low TREC levels on newborn screen.

Methods A cohort study was conducted for infants treated at Rush University Medical Center from January 2015 to April 2019 with positive

SCID screens (positive screen defined as TREC values less than 250 units/ul per statewide criteria).
Results Forty nine neonates were identified with low TREC values. 46 (94%) of these infants had repeat TREC screening, 14 (29%) of which were found to have a positive second TREC screen. Lymphocyte subsets were evaluated in 23 of these infants and 5 of which were noted to have lymphopenia (defined as absolute lymphocyte count less than 2500). 2 infants were noted to have low CD4 levels (defined as < 300 cells/ul) and 6 were noted to have low CD8 levels (defined as less than 500 cells/ul). Of note, 50% of the infants with CD4 and CD8 lymphopenia had normal repeat TREC levels. 14 of the infants were noted to have low B cell levels (defined as < 610 cells/ul). 12 infants had quantitative immunoglobulin levels and of these two were noted to have IgG levels less than 100. Of note one infant was diagnosed with partial DiGeorge syndrome via microarray. In our study population, no infants were diagnosed with SCID.
Discussion
Our study shows that testing for TREC levels on newborn screen may be beneficial in identifying not only SCID, but also other immunologic conditions. Infants in our study had evidence for both cell mediated and humoral immunodeficiency which necessitated further workup and follow up from Allergy and Immunology specialists. It may be beneficial to develop further programs to track infants identified with abnormal TREC levels on newborn screens to determine if they develop signs of immunodeficiency syndromes later in life.

(18) Submission ID#787559

A 24-Year-Old Male With Activated Phosphoinositide 3-Kinase Delta Syndrome (APDS) With Novel Deletion

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Abstract/Case Report Text
The patient was transferred to our adult clinical immunology transition clinic for low IgG and IgA, elevated IgM and B cell lymphopenia, treated with subcutaneous gamma globulin. His medical history was relevant for recurrent respiratory infections since two years-old, failure to thrive and developmental delay. He also developed chronic auto-immune hemolytic anemia (AIHA) at ten years old, accompanied by prominent lymphoid hyperplasia.
Our initial evaluation at the age of twenty years old showed massive polyadenopathy and splenomegaly. Work-up confirmed flair-up of AIHA. At that point, the diagnosis of APDS was raised. He also had mild intellectual impairment and dysmorphic features such as a mild degree of ocular depression, deep-set eyes, vaguely triangular face, small chin, but had normal stature. A customized panel for usual genes involved in classic hyperIgM syndromes, APDS, Noonan and Kabuki syndromes came back negative. At 23 years old, an urgent coloscopy was performed because of acute abdominal pain and showed diffuse ileal lymphoid hyperplasia. Biopsies confirmed reactional lymphoid hyperplasia without infection nor malignancy. A second 232 genes NGS panel associated with PID identified a heterozygote mutation in TAP1; expression of HLA class 1 was normal on flow cytometry.
The patient was then started on sirolimus for an "APDS-like syndrome" despite the lack of genetic confirmation. Six months after introduction of mTOR inhibitor, his abdominal pain had completely disappeared. TEP scan showed complete resolution of axillar, retroperitoneal and inguinal lymph nodes and significant regression of splenomegaly. A third large non-biased 6700+ genes NGS panel revealed a 30 pb de novo deletion that included the splice site of PIK3R1 exon 11 typically involved in APDS2: c.1392_1425+4del p.(Asp464Glufs*5) , which was missed by the first two panels. Indeed, oligonucleotide-selective sequencing technology used for the previous panels was associated to mapping errors of short reads and difficult detection of large deletions. Interestingly, the patient also presented some but not all dysmorphic features of SHORT syndrome which is related to PIK3R1 haploinsufficiency.
In this new era of genetic testing, this case is a reminder that we need to be aware of the pitfalls of genetic tests and that clinical judgment is still our best diagnostic tool.

(19) Submission ID#792220

Cancer in Patients with X-Linked Chronic Granulomatous Disease: A Case Report

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Abstract/Case Report Text
Intro: Patients with Chronic Granulomatous Disease (CGD) are theorized to have a lower risk of malignancy related to their lack of free radical formation. There are relatively few reports of malignancy described in patients with CGD. We report three cases of malignancies in the large cohort CGD population at the National Institutes of Health followed between 1972-2018 to add to the seven cases described in the literature.
Case 1: A 48-year-old man with X-linked Chronic Granulomatous Disease with a history of severe inflammatory bowel disease, who presented with progressive left-sided chest pain in 2017, decreased appetite and weight loss. Transthoracic lung biopsy showed atypical cells and a PET/CT showed abnormally dense mesentery and widespread hypermetabolic abnormalities. A mesenteric biopsy showed metastatic pancreatic adenocarcinoma. Palliative care was initiated. Patient expired four months after diagnosis.
Case 2: A 24-year-old man with X-linked Chronic Granulomatous Disease and severe inflammatory bowel disease requiring total proctocolectomy and who had been remotely treated with infliximab, presented in 2015 with right upper quadrant pain. Abdominal ultrasound and MRI of the liver showed multiple liver lesions. Biopsy of these lesions revealed hepatocellular carcinoma. Patient underwent two courses of radiolabeled itrium spherules. However, his disease progressed and he expired approximately five months after diagnosis.
Case 3: An 18-year-old man with X-linked Chronic Granulomatous Disease and inflammatory bowel disease who presented in 2007 with fevers, abdominal pain and pancytopenia. During the course of his hospitalization, he developed sepsis which led to his demise. On autopsy, an incidental finding of papillary thyroid carcinoma was made.
Discussion: These three patients all had poorly controlled inflammatory bowel disease. Additionally, patients with CGD are typically exposed to higher doses of radiation, leading one to expect higher rates of radiation induced malignancies. However, there are still relatively few case reports of cancer in the CGD population. Tissue biopsy is necessary for diagnosis. Due to end organ damage secondary to the underlying disease in the first two cases, treatment options were limited. Managing infections during chemotherapy can be complex due to drug interactions with chemotherapeutic agents.

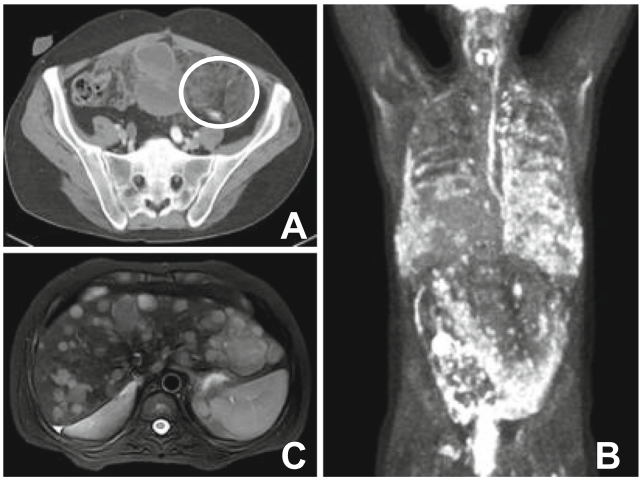

Figure 1: Clockwise from top left: A. CT imaging highlighting omental radiodensity, B. PET/CT showing extensive hypermetabolic activity, C. CT of the liver showing multiple focal lesions

(20) Submission ID#792474

A Late Presentation of MPO Deficiency

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Abstract/Case Report Text

Myeloperoxidase (MPO) deficiency is the most common inherited defect of phagocytes that impairs microbial killing since the toxicity of the respiratory burst is dampened without myeloperoxidase release from the azurophilic granules. A significant portion of these patients remain asymptomatic, however there is a clinically variable phenotype that can present if they do become symptomatic. Fungal infections with Candida strains appear to be the most frequently reported. We present an adulthood case of recurrent invasive Candidal disease due autosomal recessive myeloperoxidase deficiency from a pathogenic missense variant in the MPO gene (c.1705C>T (p.Arg569Trp)).

A 23-year-old Caucasian male was in his normal state of health without any major illnesses until 18 years of age when he was diagnosed with Candida osteomyelitis of the heel, followed by Cryptococcal meningitis the following year which ultimately required a ventriculoperitoneal shunt. In 2019, he had a prolonged hospitalization after presenting with lethargy, headache and vomiting that culminated in seizure activity and prompted an emergency room visit. Imaging at the time showed ventriculomegaly, and fluid from the shunt revealed yeast, but no bacteria. He was started on broad spectrum antifungal therapy and admitted for further management. Cerebral spinal fluid and blood cultures confirmed invasive Candida albicans meningitis. During this hospitalization, he also developed sepsis secondary to Serratia marcescens. Because of the pathogens that were being isolated, our service was consulted. Of note, our patient does not have diabetes mellitus.

A neutrophil oxidative burst assay showed an absent respiratory burst compared to control. A primary immunodeficiency panel to identify genetic variants was also sent to Invitae. Variants in CYBA, CYBB, NCF2, and NCF4 were not identified, making chronic granulomatous disease less likely.

Peroxidase staining was negative on neutrophils and normal on eosinophils, suggesting a diagnosis of MPO deficiency. This led to MPO gene sequencing for deletion and duplication analysis. A homozygous pathogenic variant consistent with a molecular diagnosis of a MPO related condition was identified. Immunoblotting of patient-derived immune cells demonstrated an absence of mature enzyme. Although not typically indicated, given the severity of his presentation, our patient remains on Fluconazole for long term prophylaxis.

His younger brother also had a history of invasive disease with Candida – osteomyelitis and meningitis. A neutrophils oxidative burst assay showed similar results in his brother and similar results with peroxidase staining, also suggesting a diagnosis of MPO deficiency. Confirmatory genetic testing has not been performed yet. Their father, who reported severe skin infections with Candida, had peroxidase stains performed on neutrophils and eosinophils which were both normal.

We have presented a patient without a significant history of diabetes mellitus who developed invasive disease from Candida and Serratia and was ultimately diagnosed with myeloperoxidase deficiency.

(21) Submission ID#793733

Systemic Juvenile Xanthogranuloma Involving the Central Nervous System Treated Successfully with ALK-Pathway Targeted Inhibition

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Abstract/Case Report Text

Introduction: Juvenile xanthogranuloma (JXG) is an often benign, histiocytic proliferative disorder of the mononuclear phagocytic system. Patients typically present with localized cutaneous lesions. Systemic disease, especially central nervous system involvement, rarely occurs but has significant morbidity and mortality risk. No standard evaluation nor therapy regimen exists for systemic JXG and little is known about the genomic alterations underlying its pathology.

Case Report: A full-term male infant presented at 4 months of age with post-prandial abdominal pain, fevers, altered mental status and weight loss. Abdominal ultrasound and CT identified renal masses. A chest CT was obtained showing a paraspinal mass with possible neural foramina extension. MRI brain and total spine was consistent with diffuse leptomeningeal disease involving the left frontal convexity, brainstem, cerebellum, and multiple cranial nerves. Abnormal enhancement was also present along the entire surface of the spinal cord extending into the cauda equina with additional enlargement of the cervical/upper thoracic cord with intramedullary enhancing masses and a right paraspinal mass. Renal biopsy yielded a pathologic diagnosis of disseminated JXG. Integrative clinical sequencing of the mass identified a somatic driving ALK rearrangement (KIF5B-ALK in-frame fusion). Tumor and matched germline DNA sequencing did not detect any alterations in the RAS/MAPK pathway. Bone marrow biopsy was negative for disease with cerebrospinal fluid analysis showing numerous monocytes and macrophages consistent with JXG.

The patient was started on therapy consisting of systemic dexamethasone, intrathecal methotrexate/hydrocortisone and systemic intravenous cytarabine. His first cycle was complicated by Pseudomonas aeruginosa

bacteremia and gangrenous cellulitis of the perianal region, treated with systemic/topical antibiotics and topical GM-CSF. Following completion of the initial cycle of therapy, the patient was noted to have declining neurologic status, including seizure-like activity. Repeat MR imaging revealed worsening CNS disease with new subdural fluid collection and progression of leptomeningeal enhancement and intramedullary cervical lesion. In light of disease progression, the decision was made to continue dexamethasone treatment, but add adjunct intrathecal cytarabine, and transition to targeted ALK inhibition via daily oral ceritinib, given its predicted CNS penetrance followed by ceritinib in combination with systemic intravenous clofarabine. Significant clinical and radiographic improvement was noted with the new targeted treatment regimen. Ceritinib therapy was tolerated well overall after a 25% dosing reduction made for initial grade 2 gastrointestinal toxicity and grade 4 hypertriglyceridemia (non-life threatening but level > 1000 mg/dL). Following continued treatment with daily ceritinib and completion of 12 cycles of clofarabine therapy, our patient experienced complete disease remission. He continues to do well on daily ceritinib monotherapy with plan to complete an additional year of therapy.

Conclusion: Our report highlights the potential benefit of real-time integrative clinical sequencing in the management of systemic histiocytic lesions, specifically non-Langerhans cell conditions. It has the potential to identify novel somatic genetic alterations, other than the typical LCH-associated BRAF mutations of the MAPK pathway, that may be therapeutically targetable. Treatment with 2nd generation ALK-inhibition in our pediatric disseminated JXG patient was a novel, biologically-rationale management approach with minimal toxicity and potentially contributed to his complete remission.

(22) Submission ID#795170

Normalization of C3 Following Bortezomib Treatment in a C3 Glomerulonephropathy Patient

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Abstract/Case Report Text

Background: C3 glomerulonephropathy (C3GN) is a progressive kidney disease with the predominant pathological feature of C3 deposits around the glomerular capillaries. C3GN patients suffer from dysregulated activation of the alternative pathway as the result of autoantibodies or congenital genetic defects that stabilize cleavage of C3. Despite therapy involving immunosuppression and complement-pathway inhibition, the prognosis for C3GN is poor. We report a patient with autoantibody-mediated, refractory C3GN who demonstrated no improvement on rituximab but achieved sustained remission on bortezomib. Follow up studies after one year demonstrated clearance of the culprit autoantibody, normalization of C3 levels, and improved pathologic appearance of the kidneys. This case supports the idea that C3GN is frequently driven by pathogenic autoantibodies that may not clear with rituximab alone. Plasma cell directed therapy has the potential to clear these autoantibodies and halt the progression of disease.

Case presentation: We report the case of a Hispanic male with chronic renal dysfunction initially diagnosed with membranoproliferative glomerulonephritis on renal biopsy at 12 years of age. Despite cellcept and prednisone, over the next 4 years, he had worsening proteinuria and an increase in protein-to-creatinine ratio. Renal biopsy suggested C3GN, and lab studies revealed a factor-H autoantibody and C3 level below the assay limit of detection. After initiating eculizumab, the proteinuria temporarily improved; however, the proteinuria eventually worsened, and he was referred to immunology.

We hypothesized that the factor H-binding autoantibody was the cause of dysregulated C3 cleavage and disease progression, and blocking the terminal complement pathway with eculizumab would not halt upstream C3-mediated kidney injury. At the age of 20, rituximab and plasmapheresis were administered to clear the factor-H autoantibody. Three months after rituximab administration, the factor-H autoantibody level decreased to the normal range, but he continued to have significant proteinuria with low serum albumin and undetectable C3 level. We concluded that the relevant autoantibody was not solely produced by differentiating memory B cells, so we decided to target the plasma cell compartment. Bortezomib was started at the age of 21, and eculizumab was continued given his initial response to treatment. After adding bortezomib, Factor H autoantibody levels dropped below prior levels and serum C3 level normalized. Renal biopsy at the age of 22 showed evidence of improving C3 deposition and less prominent glomerular hypercellularity, with stable mesangial hypercellularity, interstitial fibrosis, tubular atrophy, and sclerotic glomeruli. Although his proteinuria did not worsen, it remained persistent, suggesting that earlier introduction of bortezomib could have prevented disease advancement. There has been no further progression of kidney failure.

Conclusions: The majority of C3GN patients harbor autoantibodies to components of the alternative pathway of complement. This case provides evidence that at least some of these autoantibodies are indeed the cause of complement dysregulation, and thus are prime targets for therapy. B cell targeting therapies may be inadequate to decrease autoantibody levels for some patients. Early initiation of bortezomib, or other plasma-cell directed therapy, may effectively induce complement normalization and disease remission in these cases.

(23) Submission ID#795442

A Case Of Combined IgA And IgG Subclass Deficiency In A MECP2 Duplication Syndrome Patient

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Abstract/Case Report Text

Introduction:

MECP2 Duplication Syndrome (MDS) is a rare, X-linked genetic disorder characterized by early hypotonia, profound intellectual disability, seizures, and immunodeficiency. Combined IgA and IgG subclass deficiencies were reported to be associated with this syndrome.

Case description:

An 18-year-old male with autism, seizure disorder, infantile hypotonia, and asthma was referred to the immunology clinic for evaluation of recurrent respiratory tract infections (RTI's) and methicillin-resistant Staphylococcus aureus (MRSA) skin infections. He had poorly controlled asthma with multiple exacerbations despite treatment with high dose

inhaled corticosteroid and a long-acting bronchodilator. He has no family history of immunodeficiencies or congenital disorders.
Computed tomography(CT) chest showed bronchiectasis. His complement studies and isohemagglutinin titers were also normal. His serum immunoglobulin(IG) and lymphocytes on presentation are shown in Table 1.
He had a poor response to polysaccharide pneumococcal vaccination. He was diagnosed with combined IgG2/IgG4 subclass/IgA deficiency and was started on immunoglobulin replacement therapy and prophylactic rotating antibiotic therapy. Thereafter, his clinical course markedly improved with a reduction in the frequency of RTI's as well as the number of bronchiectasis exacerbations.
There was high suspicion for an underlying genetic disorder based on his constellation of neurodevelopment disorders and immunodeficiency. Cytogenetic evaluation with array comparative genomic hybridization(CGH) analysis showed duplication of Xq25 and Xq28 consistent with MDS.
Discussion: MDS is caused by duplications involving the MECP2 gene locus of the X chromosome at Xq28. It has a 100% penetration rate in males whereas females act as carriers and are usually unaffected. Rarely, cases of de novo mutations causing MDS have been reported. Chromosome microarray analysis is currently the best initial clinical test when MECP2 duplication syndrome is suspected. Management needs a multidisciplinary approach involving geneticists, neurologists, ophthalmologists, physical medicine and rehabilitation specialists, psychologists, gastroenterologists, and allergy and immunology specialists. Prophylactic treatment with IVIG and antibiotics has been the standard of care for immunodeficiency in these patients. Prognosis is guarded and most male patients die in the mid to late 20's because of severe RTI's secondary to immunodeficiency.
Conclusions: This case confirms the association of MDS with combined IgA and IgG subclass deficiencies. Clinicians should consider pursuing genetic evaluation for MDS in patients with neurodevelopmental disorders and immunodeficiency because the diagnosis of the syndrome can change the overall approach to management and expectations in prognosis.

Table 1. Immunoglobulin and lymphocyte studies

Serum Immunoglobulin Subclasses		Lymphocyte subset study	
IgG total	838mg/dL (normal)	CD4+ T cells	707 cells/uL (normal)
IgG1	641mg/dL (normal)	CD8+ T cells	760 cells/uL (normal)
IgG2	43mg/dL (low)	CD4+/CD8+ ratio	0.94 (inverted)
IgG3	44mg/dL (normal)	B lymphocytes	93 cells/uL (slightly low)
IgG4	2.8mg/dL(low)		
IgA	6mg/dL (low)		
IgM	114mg/dL (normal)		

(24) Submission ID#795768

Persistently Low C3 Post-Streptococcal Glomerulonephritis, Lipodystrophy, and C3 Nephritic Factor

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Abstract/Case Report Text
Introduction: C3 nephritic factor is an autoantibody that binds to the alternative pathway C3 convertase (C3bBb). This results in unchecked overactivation of the alternative complement pathway, which can lead to renal disease, partial lipodystrophy, retina disease, and frequent infections. In this case, we present a patient with partial lipodystrophy and low C3, subsequently found to have C3 nephritic factor.
Case Description: A 5 year old female presented with a 19 month history of low C3 levels. She was diagnosed 21 months ago with post-streptococcal glomerulonephritis (PSGN) after presenting with hematuria and elevated ASO titers. She had C3 levels drawn 1-2 months after time of diagnosis and C3 level was low at 27 (normal range 81-157), which was consistent with PSGN. It was rechecked 3 months after time of diagnosis and was still low. She was referred to Rheumatology at this time and was found to have a positive ANA titer 1:160. Tests for lupus and anti-phospholipid syndrome were negative. C3 normalized to the low-normal range at 6 months after time of diagnosis to 81. Her pediatrician checked to make sure it remained normal around 17 months after initial diagnosis and C3 was low again at 22. C4 was normal at 26. She was referred to Immunology for further evaluation.
During this time she was asymptomatic with no fevers, infections, hematuria, rashes, joint pain, or joint swelling. She has no history of hospitalizations other than the first for PSGN. Mother denied family history of autoimmune disorders.
Physical Exam: Physical exam was notable for abnormal subcutaneous facial fat with normal fat distribution in the rest of her body. The rest of the exam was unremarkable with normal cardiac, pulmonary, abdominal, and skin exam.
Testing:
C3 level was rechecked and low at 26. C3 nephritic factor was elevated at 1.07 (normal range 0.00-0.26). Alternate pathway complement (AH50) was confirmed twice and was undetectable, < 10 (normal level greater or equal to 46). Total hemolytic complement (CH50) was low at 22 (normal level 42-95). Other complement levels were checked and C1q, C2, C4, C5, C6, C7, C8, and C9 complement were within normal range.
Discussion: The overactivation of the alternative complement pathway by C3 nephritic factor can result in various clinical manifestations, such as C3 glomerulopathy and acquired partial lipodystrophy in predominantly the face and the upper torso. The exact mechanism of how C3 nephritic factor is related to facial and upper body lipodystrophy is not known. One proposed mechanism is that adipocytes in the face and upper body produce more Factor D, which is a complement protein utilized by C3 nephritic factor. Overactivation of the alternative complement pathway on the adipocyte then leads to formation of the membrane attack complex, resulting in adipocyte lysis.
Eye disease, such as retinitis pigmentosa and macular degeneration can develop. C3 nephritic factor can also lead to more frequent infections and renal disease. Patients need to be closely monitored. If patients develop C3 glomerulopathy, they may need to be considered for immunomodulatory therapy, such as steroids and other immunosuppressants.

(25) Submission ID#796038

Early Outcome of a Phase I/II Clinical Trial of Gene-Corrected Autologous CD34+ Hematopoietic Cells and Low-exposure Busulfan in Patients with Artemis-Deficient Severe Combined Immunodeficiency

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[15]Staff Pediatrician/Phoenix Children's Hospital
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Abstract/Case Report Text

Background: Artemis-deficient SCID (ART-SCID) represents ~3% of all SCID, but occurs in 1/2000 births in Navajo and Apache Native Americans. Artemis protein, encoded by DCLRE1C, is essential for repairing DNA double-stranded breaks, including those generated during V(D)J recombination of antigen receptor genes as T and B cells develop. Artemis-deficiency causes not only T-B-NK+ SCID, but also increased sensitivity to alkylating drugs and radiation. ART-SCID is the most difficult SCID to treat with allogeneic hematopoietic cell transplantation (HCT) due to high rates of rejection and GVHD, incomplete immune reconstitution, and toxicity following intensive conditioning regimens. As an alternative, we developed a self-inactivating lentiviral vector containing the human Artemis promoter and DCLRE1C cDNA (AProArt). We are evaluating its toxicity and efficacy in a Phase I/II gene transfer trial in ART-SCID patients.

Methods: Newly diagnosed infants with ART-SCID and older patients with insufficient immunity despite prior allogeneic HCT were eligible if organ function was acceptable. Infants needed to have no matched sibling donor and be at least 2 months old at conditioning. CD34+ cells were isolated from bone marrow or cytokine-mobilized peripheral blood, cultured with cytokines, transduced x2 with AProArt, and cryopreserved. Patients received 2 daily doses of busulfan, targeted for a cumulative exposure (cAUC) of 20mg*hr/L, with infusion of thawed cells on the following day.

Results: We treated 5 newly diagnosed infants (ART001-3&007-8) with median age 2.6m (range 2.3-3.7) and 3 previously-treated patients (ART004-6) (5.5y, 12.7y and 20.9y), with a median follow-up of 9.6m (range 1.6-17.2). The mean (SD) Bu cAUC was 19.4±1.0 mg*hr/L. Patients received a median of 6.5x106 AProArt-transduced CD34+ cells/kg (range 3.9-12.4). The average vector copy number (VCN) and transduction efficiency in the marrow grafts exceeded those in the PBSC grafts: 2.1±1.0 copies/cell vs 0.83±0.1 (p=0.02) and 75±9% vs 59±4.6% (p=0.03), respectively. There were no serious busulfan side effects. All patients had transduced peripheral blood leukocytes by 4w and 7 of 8 developed gene marking in T, B, NK and myeloid cells by 8w (Fig. 1). Gene-corrected CD3, CD4, CD4/45RA/CCR7, CD8 and CD19 cells appeared in 7 of 8 patients (Fig. 2), with ART005 having T, NK and myeloid marking without B cells at 6m post infusion. Normalization of lymphocyte proliferation to PHA occurred in the 3 evaluable (>12w) infants (Fig. 3), all 3 now outpatients off isolation. Two infants and 1 previously treated child developed autoimmune hemolytic anemia (AIHA), with 2 requiring immunosuppressive therapy. Infections included rhinovirus at presentation in ART001 that resolved with T cell reconstitution. After discharge ART001 acquired and recovered from norovirus and ART002 acquired and recovered from CMV and rotavirus. Analyses of insertion sites and T cell receptor diversity are pending.

Conclusion: Infusion of AProArt-transduced autologous CD34 cells into ART-SCID patients pretreated with very low exposure busulfan resulted in multilineage engraftment of transduced cells with evidence for T and B cell immune development. AIHA, the only complication to date, occurred early and appears to resolve following restoration of T cell immunity. These encouraging results suggest potential effectiveness of ex vivo gene therapy for ART-SCID.

Figure 1. Gene Marking (VCN/cell, VCN) in peripheral blood cell subsets post Infusion of AProArt-transduced CD34+ cells

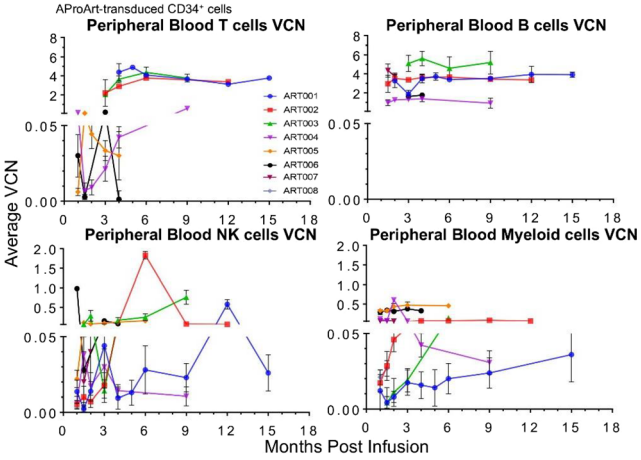


Figure 2. T & B Cell Reconstitution Post Infusion of AProArt-Transduced CD34+ cells

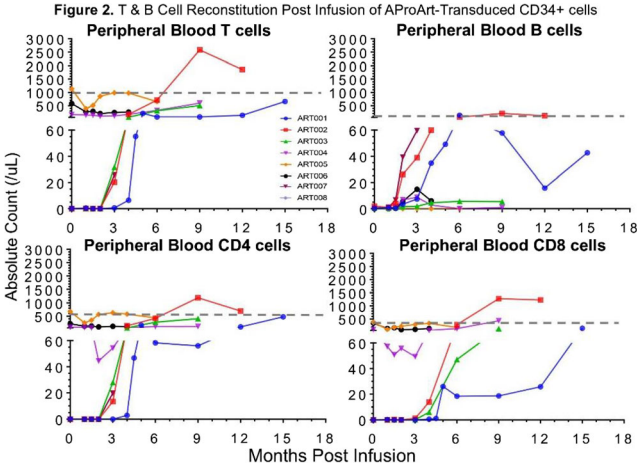
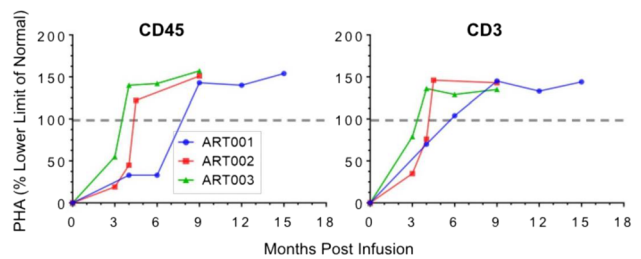


Figure 3. CD45 and CD3+ lymphocyte proliferation following infusion of AProArt-corrected CD34+ cells.



(26) Submission ID#798344

RAS-Associated Autoimmune Leukoproliferative Disorder (RALD) Accounts For A Subset Of Early-Onset Systemic Lupus Erythematosus

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Abstract/Case Report Text

Background: Systemic lupus erythematosus is a heterogeneous disorder of the immune system. Systematic genetic evaluation of patients with childhood-onset SLE (cSLE) has begun to identify phenotypic clusters of cSLE patients with classic SLE-causing genetic variants, as well as revealed unexpected genetic mimics of lupus. We report 3 patients diagnosed with cSLE with similar typical and atypical lupus features, who were subsequently found to carry pathogenic NRAS variants that are the cause of RAS-associated autoimmune leukoproliferative disorder (RALD).

Cases: All 3 patients (2 females, 1 male) presented at < 3 years of age (average age 21.7 months, range 17-27 months) with antinuclear antibodies, anti-double-stranded DNA antibodies, autoimmune hemolytic anemia, severe thrombocytopenia, antiphospholipid antibodies, hypocomplementemia and nephritis. Additionally, the patients all displayed fevers, organomegaly, lymphadenopathy and hypergammaglobulinemia. Two out of 3 patients had a malar rash, leukopenia, lymphopenia, anti-Smith antibodies, serositis or arthritis. No patient had oral or nasal ulcers or photosensitivity. Despite the fevers, lymphoproliferation and systemic autoimmunity, the patients did not display overwhelming immune dysregulation (peak ferritin 128-623 ng/mL). Interestingly, the patients were found to have monocytosis (19-45%), as has previously been reported in RALD. Double negative T cells were within normal range in the 2 patients in which this was tested. All 3 patients required aggressive immune modulation for control of their disease manifestations. Two of the 3 developed severe infections, specifically pneumococcal sepsis, during therapy. Current follow-up covers an average of 9.2 years (range 0.6 to 17 years). The patients responded to corticosteroids and were given sequential trials of various steroid-sparing therapies. In general, they appeared to benefit from both B cell depletion and T cell-directed modalities (cyclosporine, rapamycin), which are not first line therapy in cSLE. Unfortunately, 1 patient developed a fatal pulmonary infection while on treatment; her underlying disease was felt to be quiescent. Due to the early-onset of disease, each patient was selected for genetic evaluation (2 by exome sequencing, 1 by gene panel). This lead to the discovery of pathogenic NRAS variants (c.38G>A, p.G13D) in all patients, assumed to be somatic, although this was confirmed in only 1 case.

Conclusion: RAS-associated autoimmune leukoproliferative disorder can present indistinguishable from cSLE with positive autoantibodies, immune cytopenias, arthritis, nephritis and hypocomplementemia. Clinicians should consider evaluating for RALD in cSLE patients who present at an early age (< 3 years) with predominant features of lymphoproliferation and hematologic abnormalities, particularly monocytosis. T cell-directed therapy with cyclosporine or rapamycin should be considered for RALD.

(27) Submission ID#798815

Complex Multi-System Immune Dysregulation and Recurrent Infections in an Adult

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Abstract/Case Report Text

Introduction:

Cytotoxic T Lymphocyte Associated Protein 4 (CTLA4) is a crucial downregulator of immune responses. (1) Human CTLA4 loss-of-function causes dysregulation of FoxP3+ regulatory T (Treg) cells, hyperactivation of effector T cells, and lymphocytic infiltration of target organs. Patients also exhibit progressive loss of circulating B cells, associated with an increase of predominantly autoreactive CD21(lo) B cells and accumulation of B cells in non-lymphoid organs. Inherited human CTLA4 loss-of-function demonstrates a critical quantitative role for CTLA4 in governing T and B lymphocyte homeostasis. (2) This case highlights the importance of Next Generation Sequencing (NGS) in diagnosing and managing complex presentations with multi-system involvement.

Case presentation: Patient was diagnosed with diffuse large B cell lymphoma at age 22 and treated with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) in 9/2012. He underwent autologous stem cell transplant with preparative carmustine, etoposide, cytarabine, and melphalan (BEAM) in 10/2012. He subsequently developed recurrent giant condyloma acuminata following transplantation requiring 9 surgical resections, refractory immune thrombocytopenic purpura (ITP) requiring aggressive systemic steroids and high dose IVIg at least yearly, experiencing hypogammaglobulinemia, recurrent sinopulmonary infections, disseminated herpes zoster, and Kaposi sarcoma.

In 6/2014, he underwent CT/PET, revealing extensive hypermetabolic lymphadenopathy and splenomegaly. Bone marrow biopsies were negative for lymphoma, although showed a slightly hypocellular marrow (40-60% cellularity), 2% blasts, and eosinophilia without peripheral eosinophilia. Repeated evaluations for HIV, syphilis, Histoplasma, CMV, HHV6, HHV8, Bartonella, Coxciella, Brucella, HTLV, toxoplasma were negative. HTLV1 and 2 antibodies had been negative prior to transplantation. Tonsillectomy 12/2015 due to progressive enlargement showed reactive follicular hyperplasia with focal acute tonsillitis without granulomas or viral inclusions. Repeated lymphocyte enumeration and proliferation studies were normal. A repeat PET scan 3/2016 revealed persistent diffuse lymphadenopathy involving the neck, chest, abdomen, and pelvis. Left lung biopsy revealed non-caseating granulomas without lymphoma. Stains for EBV were negative. Repeat PET scan 11/2016 indicated disease progression prompting a left axillary excisional lymph node biopsy, revealing EBV lymphadenitis with large, reactive follicles with interspersed inflammation and loosely formed granulomas and CD20 positive B cells within the follicles. EBV blood PCR was negative. AFB and fungal stains were negative on all biopsies. In 1/2017, his IgG was 615 (762-1488), IgA 33 (70-390), and IgM 58 (38-328) with only 1 out of 23 protective serotypes to pneumococcus post-vaccination at 1.3 or greater. In 3/2018, IgG was 380 (762-1488).

Custom NGS panel showed a heterozygous missense variant in CTLA4 c.534c>g (p.S178R) located in the transmembrane domain. This variant of uncertain significance is suspicious and strongly suggests the diagnosis

of CTLA4 -related autoimmune lymphoproliferative syndrome . Patient was referred to the National Institute of Health, where he received a bone marrow transplant.
Conclusion:
Primary immunodeficiency diseases comprise a group of highly heterogeneous immune system diseases and around 300 forms of PID have been described. NGS has recently become an increasingly used approach for gene identification and molecular diagnosis of human diseases guiding treatment to patients who may otherwise have poor outcomes. (3)

(28) Submission ID#799299

Atypical Presentation of C8 Alpha Deficiency In A Congolese Boy

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Abstract/Case Report Text
Introduction: Inherited defects of the complement system are rare disorders that can result in unique susceptibility to infections with select bacteria. Patients with a deficiency of a complement protein early in the complement pathway (affecting C1qrs, C2, C3, Factor H, or Factor I) have increased susceptibility to infection with encapsulated bacteria, most notably Streptococcus pneumoniae and Neisseria species. In contrast, patients with a deficiency of a complement protein at the terminal end of the complement pathway (affecting C5, C6, C7, C8 alpha/beta/gamma, or C9) almost universally present with severe, recurrent, or disseminated Neisseria species infections. Most genes encoding complement proteins are found on autosomes, in which specific complement deficiencies result from biallelic mutations. Although particular complement deficiencies occur at higher frequencies in certain populations, the prevalence of specific complement deficiencies is unknown in many parts of the world, especially in underdeveloped regions, including Sub-Saharan Africa. Herein, we describe a young Congolese boy with an atypical presentation of C8 alpha deficiency.
Case Description: A 17-month-old Congolese boy with consanguineous parents (first cousins) presented with recurrent infections. Prior to an evaluation of his immune system, he was hospitalized five times. His infections included episodes of acute otitis media, bacterial pneumonia, and viral pneumonitis. A bronchoscopy revealed diffusely edematous airways and growth of Candida albicans, Moraxella catarrhalis, and Streptococcus pneumoniae in bronchoalveolar lavage cultures. Concurrently, his respiratory PCR panel was positive for adenovirus. His initial immune evaluation included assessments of his serum immunoglobulin levels, vaccine titers (tetanus, diphtheria, Haemophilus influenzae, and Streptococcus pneumoniae), neutrophil oxidative burst, lymphocyte subsets, and CH50, in which only his CH50 was abnormal (16 U/mL). His CH50 remained low on repeat assessment (16 U/mL), at which time an AH50 was pursued and also returned with a low result (1% of normal). A complement system genetic panel identified a homozygous intronic variant in C8A (c.856-12G>A). Functional confirmation of the variant revealed that the patient had a significantly decreased C8 level (18 mcg/mL) and absent C8 function.
Discussion: We present a case of a young Congolese boy with C8 alpha deficiency and a clinical presentation atypical for defects in terminal complement proteins. Our patient presented primarily with recurrent respiratory infections, including Streptococcus pneumoniae pneumonia, but without a preceding history of meningococcal disease. While it is well established that patients presenting with terminal complement pathway defects have an increased susceptibility to meningococcal disease, it is less clear if they have increased susceptibility to pneumococcal infections. Occurring between exons 5 and 6, the homozygous intronic variant in C8A identified in our patient is predicted to result in abnormal splicing. While the allele frequency of this mutation is relatively high in the African population (0.017), functional confirmation of the variant demonstrated a decreased C8 level and absent C8 function that support the pathogenesis of the mutation. The discrepancy between the allele frequency and reported disease cases could be explained in part by varying clinical manifestations seen in C8 deficiency or underrecognized disease in Sub-Saharan Africa.

(29) Submission ID#800795

Long Term Clinical Outcomes of Patients with Severe Combined Immunodeficiency (SCID) Given Bone Marrow Transplantation without Pre-transplant Chemotherapy or Post-Transplant GVHD Prophylaxis

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Abstract/Case Report Text
Rationale: SCID is a syndrome characterized by profound T, B, and (in some cases) NK cell defects that is universally fatal unless immune reconstitution is achieved. A total of 177 SCID infants have been given allogeneic bone marrow transplantation at Duke University Medical Center without pre-transplantation chemotherapy or post-transplantation graft-versus-host disease (GVHD); 90% received T cell-depleted haploidentical parental marrow and 47(26%) are known to be deceased. Post-transplantation follow-up ranged from 22 months to 37 years. The aim of this cross-sectional study is to characterize the clinical status of a large cohort of survivors treated at a single medical center.
Methods:
Clinical status was assessed by detailed questionnaires delivered by mail or electronically. Adult (≥ 18 years old) and pediatric questionnaires were based on patients' age. Patients were also contacted by telephone and evaluated at clinic visits. Molecular type of SCID, demographics, type, date and age at transplant were obtained from a clinical database.
Results:
Fifty questionnaires were completed to date from survivors ranging in age from 5 to 37 years. Twenty-nine/50 were adults ≥18 years at the time of the questionnaire. Genetic defects were known for all 50 patients- X-linked SCID was the cause in about half (Figure 1). Twenty-three of 50 patients were on immunoglobulin replacement. Thirty of 50 reported having received immunizations, and about half of those received live vaccines. Fifteen of 50 reported they were taking no regular medications; 11 reported taking prophylactic antibiotics.
>We found substantial scholastic achievement, with 17/29 adult patients reporting college attendance. Two had post graduate education including

doctorate level degrees. Occupations included physician, nurse, factory worker, musician, teacher, and engineer. One patient had 3 children. Twenty-seven /29 adult patients shared their height and weight and 78% (21/27) had a healthy BMI (BMI 18.5-24.9), while 15% (4/27) were overweight, and 7% (2/27) were underweight (< 18.5). In pediatric patients, the average age and sex-adjusted BMI was at the 47th percentile and only 3 had a BMI that was < 5th percentile. Thirty-four/50 patients reported seeing an immunologist regularly. In the adult group, 38% reported no longer seeing an immunologist.

The health conditions reported were similar to those common in the general population, and included rashes, warts and mouth ulcers. Most reported these were transient, self-resolving issues. Thirteen of 50 (26%) reported having ADHD, higher than NIH reported rates which estimate ADHD in 8.1% of adults and 11% of children). Ten of 50 (20%) reported having anxiety, similar to the NIH reported prevalence of 19.1% in the general population. 34/50 (~68%) reported having no active concerns about their health.

Conclusions:

Overall, our findings are consistent with those in the last update done by Railey et al, J. Peds. 155:834-840, 2009 in this population. Patients are doing well with most problems similar to those common in the general population. Most have a healthy BMI. ADHD had a higher prevalence than in the general population. More than 1/3 of SCID patients are not seeing an immunologist regularly, and a majority do not have any active concerns.

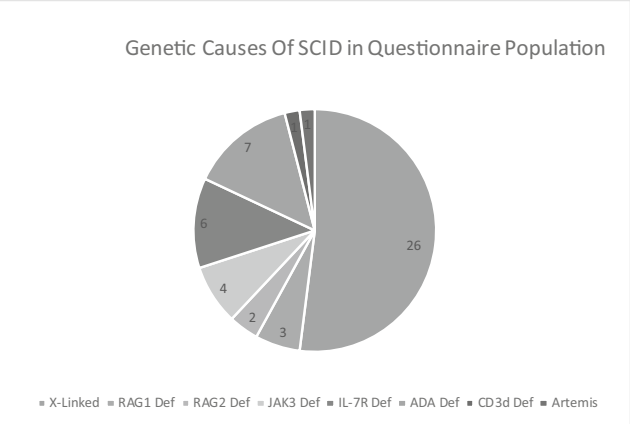

Figure 1. Genetic Causes of SCID in the 50 patients whose questionnaire data are presented. X-linked SCID was the most common, followed by ADA and IL-7R deficient SCID

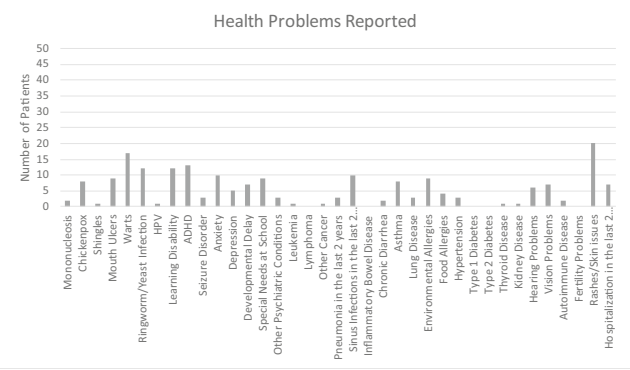

Figure 2. Health conditions reported in 50 SCID patients who received a bone marrow transplant without conditioning.

(30) Submission ID#801617

Frequency of Myopathy in Primary Immunodeficiency: Data from the USIDNET Registry

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Abstract/Case Report Text

Rationale: Myopathy has been occasionally documented in patients with primary immunodeficiency (PID). However, data on frequency and patient characteristics associated with myopathy are lacking. We performed a descriptive analysis of patients with primary immunodeficiency (PID) in the USIDNET having myopathy as a feature of their primary disease.

Methods: The USIDNET registry was queried for the spectrum of myopathic disorders in PID patients that had been entered into the registry as of November 26, 2019.

Results: A total of 63 PID patients with myopathy were identified, 36 of which (57.1%) were female. Median age at onset of symptoms related to PID was 4 years (range 0.1-72 years, IQR 0.5-25 years). Median age of diagnosis of PID was 8.3 years (range 0.4-77 years, IQR 3-32.5 years). Age at onset of myopathic disorders was not known. Twenty-eight (44.4%) patients had a diagnosis of common variable immunodeficiency (CVID), 6 (9.5%) had agammaglobulinemia, 3 patients each (4.8%) were diagnosed with severe combined immunodeficiency, hypogammaglobulinemia, or 'HLH and pigmentary disorders', while 2 patients (3.2%)were reported in each category of combined immunodeficiency (CID), autoimmune lymphoproliferative syndrome (ALPS), and autoinflammatory disease. Thirty-five patients (55.6%) had a causative gene variant identified attributable to PID. The most common variant identified was BTK (6 patients) followed by AIRE, LYST, CYBB (3 patients each) and PI3KCD (2 patients). Eighteen individual patients had other variants identified (Figure 1). 15 patients had cellulitis or skin/subcutaneous tissue infection, 7 patients had a 'skin or subcutaneous tissue abscess', 2 had pyoderma gangrenosum, and 1 patient with eczema herpeticum. Within this cohort of 63 patients, the most common myopathy listed was myositis (18) followed by 'muscle weakness' (11), dermatomyositis (8), myalgia/s (6), myalgia/myositis (4), myopathy (4), polymyositis (2), steroid-induced myopathy (2). No patient had an infectious myositis or muscle abscess listed. Eighteen patients (28.6%) had a myopathic disorder at the time of diagnosis of their PID. Thirty-one patients (49.2%) received prednisone, 11 (17.5%) received hydrocortisone and 2 (3.2%) received dexamethasone. Two patients had a diagnosis of adrenal insufficiency. Nine patients (14.3%) underwent hematopoietic stem cell transplantation. Nine patients (14.3%) died; median age of death 16 years (range 0.4-40.6 years, IQR 3.6-21.1 years).). One patient with chronic granulomatous disease had myopathy due to Duchenne muscular dystrophy, which was listed as a cause of death. No other myopathic disorders were listed as a cause of death for the other patients.

Conclusion: Myopathy and inflammatory myopathic disorders occur at relatively high frequency in PID, and may be present even at the onset of clinical symptoms. The underlying etiology can be speculated to be multifactorial. Further subgroup analysis is warranted to elucidate possible variant-specific or treatment-associated characteristics of myopathy in PID.

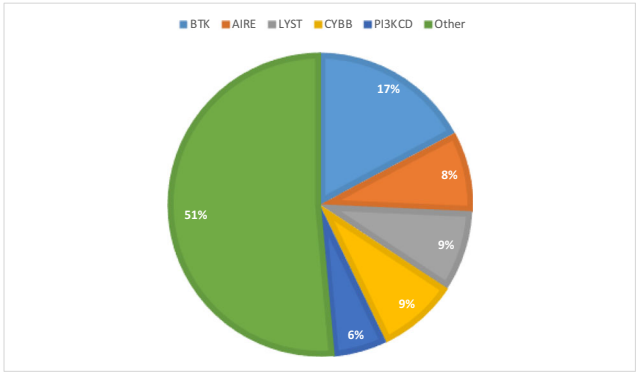

Figure 1 Frequency of monogenic variants identified from 35 patients with myopathy having gene data in the USIDNET PID registry. Each Individual patient in the 'Other' Category (n=18) had the following variants identified: *RAG2, STAT1, PI3KR1, CLTA4, FOXP3, IKBKG, MAP3K14, UNG, ATM, DOCK8, PGM3, PNP, IL2RG, IL7RA, IRF2BP2, TNFRSF13B, TNFRSF1A,* and *MEFV* (Figure 1).

(31) Submission ID#801755

Clinical Manufacturing and Banking of Virus-Specific T cells

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Abstract/Case Report Text

Virus specific T cells (VST) have shown promising clinical efficacy in patients with viral infections after hematopoietic stem cell transplant. Despite being used to treat over 500 patients worldwide, critical quality attributes and parameters associated with successful VST products have not been established. Here we report in depth characterization and parameters established from a total of 81 VST products consisting of either trivirus (65 products targeting CMV, EBV, adenovirus) or hexavirus (16 products targeting CMV, EBV, adenovirus, HHV6, parainfluenza, BK virus) VSTs. We define a successful VST product as meeting release criteria (>70% viability, < 5.0 EU/kg endotoxin, 10 spots above background/100,000 cells). A median of 76 million (range: 26-404) peripheral blood mononuclear cells (PBMCs) were isolated from a median of 54mL (range: 17-100) whole blood from 81 consenting donors. Clinical-grade VSTs were initiated using a median of 29 million (range: 8-80) PBMCs, stimulated with overlapping peptides derived from viral antigens (CMV (IE1 and pp65), EBV (LMP2 and EBNA1), Adv (Hexon and Penton) +/- HHV-6B (U54 and U90) +/-BKV (LgT and VP1) +/-HPIV3 (Matrix and NP)) at 200ng/μL/peptide, and cultured in G-Rex devices for 10-12 days. Clinically frozen cells yielded a median of 234 million cells (range: 35.5–780) with a median of 96% (80-100%) viability. 70% of triviral VST products targeted all 3 viruses by ELISPOT assay, 25% targeted 2, and 6% targeted 1. 19% of Hexaviral VST products targeted all 6 viruses, 31% targeted 5, 19% targeted 4 and 2, and 6% targeted 1 and 3. A median of 68% (range: 17-95%) were CD4+ T cells, 27% (83-4%) CD8+ T cells, and 0.4% (4-0%) CD16+CD56+CD3- NK cells. Sixty-five products met criteria for clinical release. To date, 38 patient-specific VST products have been infused, and 46 third-party VST infusions have been given from 19 products. In summary, VSTs were successfully manufactured from all donors, and all patient-specific products facilitated at least 1 dose, independent of donor white blood cell counts. Parameters associated with the highest expansion, specificity and in vivo potency are currently being evaluated.

(32) Submission ID#802030

Refractory Autoimmune Hemolytic Anemia Successfully Treated With Daratumumab in a Patient with MHC Class II Deficiency

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Abstract/Case Report Text

Introduction: Bare lymphocyte syndrome (BLS) type II complementation group B is a combined immunodeficiency caused by biallelic mutations in RFXANK that result in the absence of major histocompatibility complex (MHC) class II proteins on the surface of leukocytes. MHC class II deficiency presents with vulnerability to infection, systemic autoimmune disease, and, specifically, refractory autoimmune cytopenias. We present a patient with BLS type II complementation group B whose autoimmune enteritis and refractory, life-threatening autoimmune hemolytic anemia (AIHA) were successfully treated with daratumumab (an anti-CD38 IgG1κ human monoclonal antibody).

Case Description: After immigrating to the United States, a young boy of Moroccan ancestry established care for episodic severe AIHA. His episodes were characterized by jaundice, fatigue, and pan-reactive autoantibodies, which required a combination of blood transfusions, corticosteroids, and mycophenolate mofetil to control. His family history revealed a sister with BLS type II complementation group B. Genetic testing confirmed that he too was homozygous for c.338-25_338del26 (752delG-25) in RFXANK – a founder event seen in North African populations and the most frequent mutation responsible for MHC class II deficiency. Additionally, a younger brother was confirmed to carry the homozygous mutation. Throughout puberty, his AIHA episodes increased in frequency and severity and were increasingly refractory to immunosuppressive therapy including high-dose corticosteroids, mycophenolate mofetil, sirolimus, rituximab, bortezomib, and high-dose intravenous immunoglobulin. Eventually, he required prolonged hospital admissions and daily blood transfusions, during which time he developed severe infections, including recurrent Salmonella enterica bacteremia, polymicrobial gastroenteritis, and recurrent herpes simplex virus-1 gingivostomatitis. Splenectomy was performed at 16 years of age, resulting in a 6-month clinical remission. However, transfusion-dependent AIHA returned in addition to autoimmune enteritis manifesting as diarrhea, malabsorption, and GI bleeding. As alternative management for AIHA, he was initiated on daratumumab. Following four weekly doses of daratumumab, his hemoglobin normalized and enteritis resolved.

Discussion: Autoimmune cytopenias are a frequently encountered manifestation of many primary immunodeficiency diseases, including MHC class II deficiency. Non-specific immunosuppressive agents may maintain stability of disease, but risk further susceptibility for infection. Furthermore, diseases may become refractory to typical treatment regimens. Here, we describe a patient with BLS type II complementation group B who presented atypically with moderate AIHA, only worsening

to severe AIHA and autoimmune enteritis after puberty. In comparison, the patient's affected sister died from complications of infections and autoimmune disease in the first decade of life, and his younger brother underwent hematopoietic stem cell transplantation (HSCT) for similar complications at 3 years of age. Following a series of failed treatment regimens and without an adequate HSCT donor available, his AIHA responded to the anti-CD38 monoclonal antibody daratumumab (FDA-approved for treatment of multiple myeloma). Non-malignant plasma cells also express CD38, which has been the basis for using daratumumab to treat AIHA, particularly in severe cases post-HSCT. This is the first reported case of successful treatment of AIHA with daratumumab in a patient with MHC class II deficiency, suggesting it may be a reasonable treatment option in MHC class II deficiency patients and perhaps other primary immunodeficiency diseases with refractory AIHA.

(34) Submission ID#802398

Clinical Characteristics And Infectious Complications In Patients With Rare Forms Of Syndromic Immunodeficiency: Data From The USIDNET Registry

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Abstract/Case Report Text

Background: The spectrum of clinical characteristics, infectious complications, and immunologic abnormalities in rare forms of syndromic immunodeficiencies is not well defined. Large registries can improve our understanding of these rare diseases.
Methods: The US Immunodeficiency Network (USIDNET) Registry was utilized to identify 784 patients with syndromic immunodeficiency. DiGeorge syndrome (64.2%) was the most prevalent, followed by hyper-IgE syndrome (13.4%), Wiskott-Aldrich syndrome (12%), ectodermal dysplasia with immunodeficiency (3.7%), ataxia telangiectasia (3.6%), undefined syndromic immunodeficiency (1%), Omenn syndrome (1%), CHARGE syndrome (0.7%), and dyskeratosis congenita (0.2%). As the focus of this study was to investigate rare forms of syndromic immunodeficiencies, data was analyzed excluding the well known example of DiGeorge syndrome.
Clinical characteristics: Out of the 281 patients with a diagnosis of syndromic immunodeficiency other than DiGeorge syndrome, 66% (n=185) were male and 34% (96) were female. The average age of disease onset was 1.9 years and average age of diagnosis was 5.8 years. Diagnostic delay was most prominent in Hyper IgE syndrome, where the age of onset was 2.9 years and age of diagnosis was 9.3 years. On the other hand, CHARGE syndrome was diagnosed at a mean age of 0.1 years.
Infectious complications and malignancy: A total of 1782 reported infectious conditions were sorted by organ system. Pulmonary infections were the most common (27%, n=478) followed by skin (26%, 470), ENT (21%, 381), gastrointestinal (8%, 147), systemic (8%, 145), genitourinary (2%, 42), CNS (2%, 33), oral (2%, 32), and musculoskeletal (2%, 30) infections. Pneumonia was the most commonly reported pulmonary infection (60%, n=285). Of all ENT infections, otitis media (44%, n = 167) was the most common, followed closely by sinusitis (42%, 160). A total of 943 infectious pathogens were recorded, of which 21% were Staphylococcus aureus (n=194), 13% Candida (123), 7% Pseudomonas (68), 6% Aspergillus (55), 5% common respiratory viruses (47), 4% other Staphylococcus (34), and 4% Streptococcus pneumoniae (34). Malignancy was diagnosed in 14% of patients (n=39), of which 51% (20) were lymphoma.
Therapy: Immunoglobulin replacement therapy was reported as done in 58% (n=162), not done in 35% (97), and was not reported in the rest of the cohort. Twenty-three percent of the cohort underwent stem cell transplant (n=66), with the highest rates in Omenn syndrome (7/7 patients, 100%) and the lowest rates in ataxia telangiectasia (0/27, 0%) and undefined syndromic immunodeficiency (0/8, 0%).
Conclusions: USIDNET provides a resource to describe the clinical characteristics of patients with rare forms of syndromic immunodeficiency. Data from the registry shows that these patients are susceptible to a wide variety of infectious conditions and pathogens. In this cohort, more than half received immunoglobulin replacement therapy and about a quarter received stem cell transplant. The overall diagnostic delay is about 4 years. This suggests the importance of immunological investigations in patients with congenital anomalies, syndromic features, or chromosomal aberrations who have recurrent infections.

(35) Submission ID#802527

Dedicator of Cytokinesis 8 (DOCK8) Deficiency Presenting In a Preterm, 24-Week Gestational Age Infant

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Abstract/Case Report Text

INTRODUCTION: DOCK8 deficiency (autosomal recessive hyper IgE syndrome) may present with features of combined immune deficiency (CID) early in life. Here we report a premature male infant of 24 weeks gestation who had generalized skin candidiasis within 2 weeks after delivery – the youngest patient with DOCK8 deficiency ever identified by genetic testing.
CASE HISTORY: The infant was admitted to the neonatal intensive care unit for extreme prematurity (birthweight 585 g, vaginal delivery), respiratory failure, and possible sepsis. He was the first born to non-consanguineous parents without a family history of immunodeficiency. The infant was placed on a ventilator and given antibiotics for possible sepsis. During the first 2 weeks, candida skin infection was diagnosed (with a positive KOH preparation) and treated successfully with topical nystatin. Herpes simplex virus PCR of the skin and blood cultures for bacteria and fungi were negative. Subsequently, the infant developed conditions commonly associated with prematurity, including anemia, hyperbilirubinemia, patent ductus arteriosus, cardiac instability, acute urinary tract infection, and bilateral grade I intraventricular hemorrhages. Weight and length trended at 3%. Occurrence of severe candida infection with scalded skin and intractable unconjugated hyperbilirubinemia prompted whole exome sequencing (collected on day 10; reported on day 30). Results showed two heterozygous pathogenic DOCK8 variants, compatible with autosomal recessive hyper IgE syndrome: c.1963C>T (p.Gln655*) and a ~33.39 kb DOCK8 deletion (exon 1 and intron 1). The mother carries the p.Gln655* variant. Testing for the ~33.39 kb deletion in parents is pending, to confirm biallelic DOCK8 variants in the infant. Immune laboratory studies included: normal newborn Trec screening; normal IgG at 12 weeks (76 mg/dL), and borderline low T and B cells at 5, 9, and 12 weeks (PMIDs 31220471, 27548364) with an increasing trend (CD4+ T cells: 944 -> 965-> 1581; CD8+ T cells: 396 -> 519 -> 737; B cells: 234 -> 878 -> 1358). CD4 phenotypes at 9 weeks (by

another laboratory) showed normal distributions of various subtypes including naive, memory (central and effector), and regulatory T cells.
DISCUSSION: Diagnosis of DOCK8 deficiency so early in life presents a unique opportunity for anticipatory guidance and treatment. A report (25724123) on 64 patients (median age 10 years) described bacterial, viral, and fungal infections in 70-84% of patients, low IgG levels in 3%, low IgM levels in 62%, low CD4 and CD8 in 29%, and a mean IgE level of 5,201 IU/mL. The infant's clinical course has been otherwise similar to courses seen for same gestational age infants. T and B cell counts were on the low side but followed an expected developmental pattern (27548364). Infection prevention with antibiotic prophylaxis (viral, fungal), IVIG, and immunizations (avoiding live viral vaccines) are needed to prevent early morbidity and improve the success of hematopoietic stem cell transplantation (30466772). Interferon-alpha 2b has shown efficacy against severe viral infections (24743019). The patient may be a strong candidate for HSCT, because a recent review reported a high survival rate, especially when done before 8 years of age (98% vs 78%; 30391550).

(36 Submission ID#802725)

First Identified Case of SCID In Puerto Rico After Implementation Of Mandatory Newborn Screening Test: A Case Report

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Abstract/Case Report Text
Background: Severe combined immunodeficiency (SCID) is an infantile-onset primary immunodeficiency in which there is dysfunction of T-cells, B-cells and NK cells. Although fatal without treatment, newborn screening test (NBS) has made it possible to detect SCID before affected infants experience severe opportunistic infections and complications. Currently, NBS is mandatory in all states of United States (US) including Puerto Rico (PR). Before implementation of the screening, a pilot program in 2011 identified one patient with SCID in PR that was successfully transplanted. After implementation of the mandatory test in August 2015, a total of 103,680 newborns in PR were screened with one case identified and confirmed in October 2019.
Purpose/Objective: Report and describe the data of the first SCID patient diagnosed in October 2019 after implementation of the mandatory NBS test for SCID in PR.
Method: Retrospective record review of data, laboratories, treatment and management of the first SCID patient that was identified with NBS test at PR.
Results: The patient had a positive NBS result based on a Real Time PCR analysis for TREC. Two samples showed undetermined Ct values for TREC amplification indicating the absence of T cells. Reference gene amplified as expected. Initial immunology evaluation with lymphocyte subset panel was performed. Results showed the following: CD3: 4 cells/uL (2%), CD4: 1 (1%), CD8: 4 (2%), NK: 8 (4%), CD19: 167 (90%) and CD45RA was not reported. Patient laboratory results consistent with T -, B+, NK- SCID. Genetic PID panel was done. One pathogenic variant, c.670C>T (p.Arg224Trp) in IL2RG that is consistent with a diagnosis of X-linked SCID was identified. Another three genetics variants were found; c.1356+4C>T, in CARD14, c.3293T>C (p.Ile1098Thr), in DOCK2 and c.981C>G (p.Asp327Glu), in ZAP70. All of them with uncertain significance. CXR showed peri-bronchial thickening concerning for viral pneumonia. Patient was admitted to hospital, isolated and placed on empiric Cefepime treatment pending blood cultures and repeating images. He received IVIG and prophylactic doses of TMP/SMX, acyclovir, azithromycin and fluconazole. Repeat CXR was found to be clear. Cefepime was discontinued once blood cultures had been negative. During hospitalization patient developed one episode of bloody stools for which infectious workup was done. Changing in newborn formula resolved patient symptoms. Case was presented to transplant centers in US and options were broadly discussed with parents. Bone marrow transplant versus gene therapy were considered. Parents elected gene therapy at Saint Jude Children's Hospital. At present, patient is pending transfer for gene therapy. With this confirmed case, we estimate an incidence in PR to be 1:100,000 from 2015 to 2019. Overall incidence is consistent with US and represents the first case in the last 4 years that was identified and confirmed in PR.
Conclusion: We identified one infant with abnormal TRECs that subsequently lead to the diagnosis of X-linked SCID. Mandatory implementation of NBS for SCID in PR provided the opportunity to successfully recognize, manage and define best treatment options for this patient. Early detection creates the opportunity to provide immune reconstitution with better outcome while infants are healthy and uninfected.

(38) Submission ID#802879

A Novel Immunodeficiency Disease Associated With A Congenital Disorder Of Glycosylation: MAN2B2 Deficiency

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Abstract/Case Report Text

Congenital disorders of glycosylation (CDGs) are genetic disorders characterized by heterogeneous clinical features, often including immune dysregulation. Here, we present the first case of autosomal recessive deficiency of the mannosidase gene MAN2B2, manifesting as syndromic combined immune deficiency with immune dysregulation.

A female child born to consanguineous parents, suffered from recurrent pneumonias, thrush, small vessel vasculitis and arthritis since early in infancy. At 16 months of life, she had a thrombotic stroke and left hemiparesis. Psychomotor developmental delay was noted. She continued to have recurrent flares of vasculitis and arthritis, multiple respiratory infections that required intubation, and chronic diarrhea requiring total parenteral nutrition. Physical exam revealed microcephaly, low height and weight, strabismus, beaked nose, hyper-extensible skin, pectus carinatum, and mild hepatomegaly. She had significant speech delay and was only able to walk with support.

Laboratory studies at 4 years revealed normal IgG/IgA/IgM but markedly elevated serum IgE (42,550 kU/L), anemia (Hb 8.7 g/dL), thrombocytopenia (78 x109/L) and lymphopenia (520 cells/L), with low T and B cell counts, very low proportion of naïve T cells, skewed repertoire of CD8+ T cells, undetectable TREC levels, and impaired T cell proliferation to mitogens and antigens. There was an elevated percentage of circulating plasmablasts (7.4%) and of dysreactive CD21low CD38low B cells (53.8%). At the age of 5, HSCT with reduced intensity conditioning was performed from her phenotypically HLA-matched father, with improvement of T and B cell count and function.

Whole exome sequencing (WES) identified a homozygous missense variant in the Mannosidase alpha class 2B member 2 (MAN2B2) gene (p.Asp38Asn), that segregates with disease in the pedigree. The MAN2B2 Asp38 residue is evolutionary conserved. The p.Asp38Asn allele has a minor allele frequency of 0.0002687 in gnomAD, with no homozygotes. The CADD score for this variant is 28.300, significantly higher than the Mutation Significance Cutoff score (3.313).

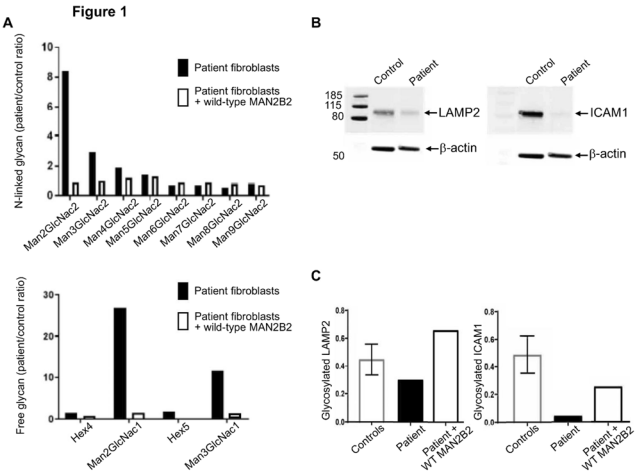
MAN2B2 is involved in the lysosomal degradation of glycoproteins and demannosylation of free N-glycans. In particular, MAN2B2 cleaves Man2GlcNac1 to generate Man1GlcNac1.

Serum N-glycan profiling revealed elevated Man5/Man6 and Man5/Man9 in the patient. N-linked and free glycan profiling by mass spectrometry (MS) showed accumulation of Man2GlcNac2, Man2GlcNac1 and Man3GlcNAc1glycans in patient fibroblasts as compared to control cells, consistent with defective lysosomal glycoprotein degradation.

Lentiviral transduction of wild-type MAN2B2 into patient fibroblasts led to normalization of the N-linked glycan profile, with reduction of Man2GlcNac2 from 8.4 to 0.9 times control levels, and of Man2GlcNac1 from 26.9 to 1.5 control levels, indicating rescue of the impaired deglycosylation (Figure 1A).

Western-blotting demonstrated defective N-glycosylation of LAMP2 and ICAM1 proteins in patient fibroblasts (Figure 1B), which were corrected upon lentiviral transduction of wild-type MAN2B2 (Figure 1C). Overall, our results indicate that loss of MAN2B2 enzymatic activity leads to dysregulation of deglycosylation and abnormal mannosylation of glycans.

In conclusion, we have demonstrated that MAN2B2 deficiency accounts for a novel autosomal recessive CDG with prominent features of immune deficiency and immune dysregulation.



(39) Submission ID#802956

Heme Oxygenase-1-Deficiency with Asplenia, Recurrent Infections, and Interstitial Pulmonary Fibrosis

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Abstract/Case Report Text

Heme oxygenase-1 (HMOX1) is a rate-limiting enzyme that catalyzes the degradation of heme to carbon monoxide, ferrous iron, and biliverdin, which becomes bilirubin. These byproducts are implicated in inflammation, cell homeostasis, and antioxidant defense(1). HMOX1-deficiency is an extremely rare autosomal recessive disorder with a complex presentation of a wide spectrum of symptoms, including hemolytic anemia and hyperinflammation, requiring genetic testing for confirmed diagnosis(2). We report the fifth known case of HMOX1-deficiency(3-5), a boy who presented at 4 years of age with aspects of the characteristic phenotype, but also had early onset asplenia, interstitial lung disease, and previously undocumented immune deficiency.

Patient's presentation was notable for hyperinflammatory exacerbations triggered by viral and bacterial infections as well as vaccinations. Episodic flares occurred every few months lasting weeks to months with fevers of 102-104F, hypoxia, leukocytosis above 40,000/mm3, hemolytic anemia with negative Coombs, thrombocytosis exceeding 1 million/mm3, transaminitis, hemoglobinuria, hyperferritinemia to 4,000ng/mL, and elevated LDH to 28,000IU/L.

Immune evaluation revealed normal immunoglobulin levels and adequate vaccine titers to both protein and carbohydrate antigens. Although class switched populations were normal, B-cell phenotyping showed absent immature and transitional B-cells, low mature memory, and reduced CD27+ memory B-cells at 6% (normal >8%). Mitogen stimulation with phytohemagglutinin and anti-CD3 were decreased (24.7% of control and 21.5% of control, respectively). T-cell phenotyping demonstrated CD4 population heavily skewed to immaturity with 65% of cells with naïve

phenotype CD45RA+CD27+CCR7+. There were few effector-memory T cells and the CD8 population was skewed towards immaturity with >65% of the cells naïve.
Liver biopsy was performed secondary to hepatomegaly yielding mild to moderate sinusoidal fibrosis. Bone marrow biopsy revealed a normocellular marrow with 3% blasts, increased megakaryocytes, and extensive hemophagocytosis. Natural killer cell function was very low, while soluble IL-2Ra level was normal. Further workup for hemoglobinopathies, metabolic defects, congenital disorders of glycosylation, lysosomal storage disorders, Wilson's disease, autoimmune hepatitis, inherited and autoimmune hypercoagulability disorders, connective tissue disorders, myositis, and myopathies were all unremarkable. Imaging demonstrated asplenia and Howell-Jolly bodies were present. Hemophagocytic lymphohistiocytosis (HLH) genetic testing showed no variants.
He was suspected to have systemic juvenile idiopathic arthritis (SoJIA) with episodes of macrophage activation syndrome. The frequency of his autoinflammatory flares increased such that he was corticosteroid dependent by age 9, having failed methotrexate, azathioprine, and anakinra. He was started on tocilizumab with laboratory improvements, but his lung disease progressed and became oxygen dependent. Lung biopsy confirmed nonspecific interstitial pneumonitis (NSIP) with cholesterol granulomas also seen in SoJIA. Ultimately, chronic lung disease led to his death at age 10. Whole exome sequencing yielded a paternal frame shift HMOX1 and maternal splice donor HMOX1 resulting in absence of protein. Bone marrow transplantation (BMT) in HMOX1 deficient mice have rectified phagocytotic defects and thereby their autoinflammatory phenotype, but no human reports for BMT treatment of HMOX1-deficiency has been described. Here we describe a phenotype expansion for HMOX1-deficiency to include not only asplenia and hepatomegaly, but also interstitial lung disease with cholesterol granulomas and inflammatory flares.

(40) Submission ID#802980

Biologics in STAT3 Loss-of-Function Hyper IgE Syndrome: A Report of 3 Cases Demonstrating Improved Clinical Outcomes

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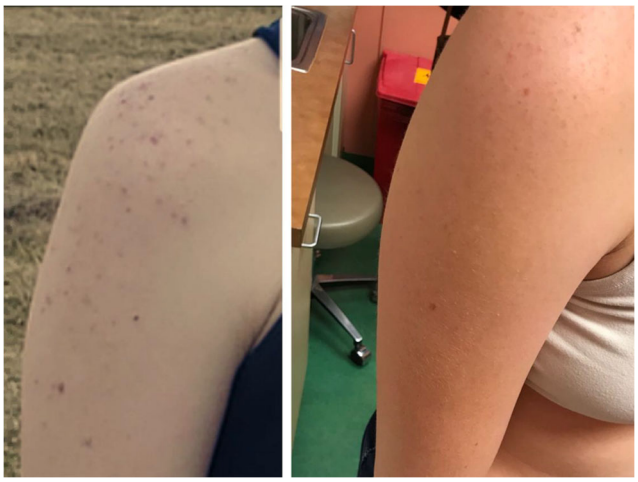
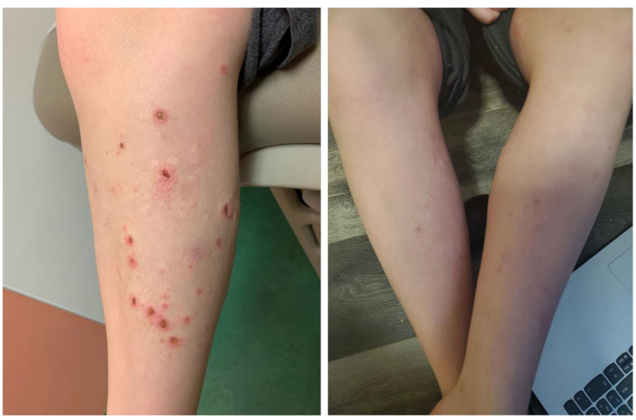
Abstract/Case Report Text
Introduction: Mutations in the gene encoding signal transducer and activator of transcription 3 (STAT3) cause autosomal dominant hyperimmunoglobulin E syndrome (AD-HIES) characterized by recurrent skin and sinopulmonary infections, atopic dermatitis, and elevated serum immunoglobulin E (IgE) levels. Treatment is largely aimed at controlling symptoms and preventing infections with no standard of care. There is a paucity of literature describing the utilization of biologic therapies in the AD-HIES patient population. We present 3 patients from one family with AD-HIES successfully treated with monoclonal antibody therapies targeted at Il-5, Il-4 and Il-13.
Case Descriptions:
Patient 1: 13-year-old female with STAT3 LOF c.1003C>T (p.Arg335Trp) with a history of atopic dermatitis and asthma requiring 2-5 steroid courses per year with frequent school absences. She developed a severe pruritic rash covering her upper body 18 months ago that failed to respond to antihistamines and topical antibiotics prescribed by her primary care provider. Given her poorly controlled asthma and our concern for a follicular type morphologic variant of atopic dermatitis, dupilumab was initiated. Her scoring atopic dermatitis (SCORAD) prior to initiation of biologic therapy was 53.8 and improved to 4.5 following 12 doses (24 weeks) of dupilumab with clear dramatic improvement in her skin and quality of life (Figure 1). She also reports decreased asthma severity with no steroid courses, reduced albuterol usage, and significant decline in school absences since initiation of dupilumab.
Patient 2: 17-year-old female with STAT3 LOF c.1003C>T (p.Arg335Trp) who is the sister of Patient 1. She has a history of severe asthma requiring frequent emergency department visits, hospitalizations, and 4-5 steroid courses per year despite therapy with high-dose fluticasone-salmeterol. Spirometry prior to April 2017 demonstrated an obstructive pattern with an FEV1 ranging from 51-64%. She was initiated on mepolizumab in April 2017. Subsequent spirometry demonstrates an FEV1 average of 115% with a range of 96-126%. She had one hospitalization in early 2018 but otherwise no hospitalizations for asthma since initiation of biologic therapy.
Patient 3: 15-year-old female with STAT3 LOF c.1003C>T (p.Arg335Trp) who is the paternal first cousin of Patients 1&2. She has a history of severe atopic dermatitis with associated pruritus and picking behaviors, poorly controlled despite daily triamcinolone application. She previously failed ultraviolet therapy and crisaborole. She also has a history of severe asthma requiring 2-5 steroid courses per year despite high-dose fluticasone-salmeterol. Dupilumab was started in May 2019. SCORAD prior to initiation monoclonal antibody therapy was 80.3 and declined to 21.2 following 13 weeks (7 doses) of dupilumab therapy with marked improvement in skin appearance and pruritus (Figure 2).
Discussion:
We present three cases of AD-HIES caused by STAT3 loss-of-function mutations treated successfully with monoclonal antibody therapies targeted at Il-5 or Il-4 and Il-13. To the best of our knowledge, there is no published data describing the use of these biologic agents in the treatment of AD-HIES. Future studies are needed to clarify the role of these cytokines in the pathogenesis of AD-HIES and to elucidate clinical indications for biologic therapy in this patient population.





Informed consent was obtained from all individual participants included in the study.

(41) Submission ID#803650

A Case of a Novel Presentation of CTLA-4 Haploinsufficiency

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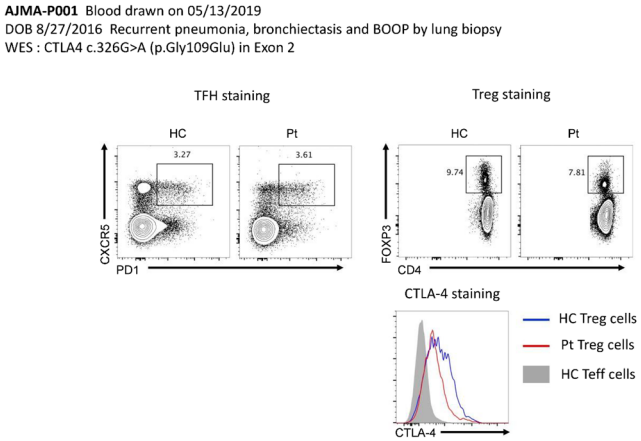
Abstract/Case Report Text

CTLA-4 is a potent inhibitor of T cell proliferation that competes with costimulatory receptor CD28 for its ligands CD80 and CD86 expressed on antigen presenting cells. Heterozygous loss-of-function mutations in CTLA-4 have been identified in patients with lymphocytic infiltration of multiple nonlymphoid organs (Lo et al). The patient is a 2-year-old Jordanian male born at term to nonconsanguineous parents, hospitalized at 1 mo for LLL pneumonia, and at 2 mo and at 4 mo he was evaluated in the ED and diagnosed with non RSV-bronchiolitis with LLL infiltrate thought to be secondary to atelectasis. At 2 yo he developed LLL pneumonia and respiratory failure requiring PICU admission. He was treated with ceftriaxone. After discharge, he had 6 weeks of intermittent fever, progressive fatigue, productive cough, and FTT. He received courses of cefdinir, clindamycin, and TMP-SMX without improvement. Chest CT revealed left lung consolidation, LLL bronchiectasis, LUL tree in bud opacities, and hilar lymphadenopathy. Bronchoscopy with BAL revealed no bacterial growth and no acid-fast bacilli. 16srRNA NGS was positive for H. influenzae. Lung biopsy demonstrated acute and chronic bronchiolitis with bronchiolitis obliterans and intraluminal polyps, with lymphocytic infiltration involving the bronchi and bronchioles. The lung parenchyma showed airspace filling with foamy macrophages and chronic interstitial inflammation. Acid fast and fungal stains were negative. He was treated with systemic steroids for bronchiolitis obliterans with noted improvement. The severity of lung disease at such an early age prompted an immune evaluation. Sweat test, ANCA, anti-PR3 and HIV were negative. Total immunoglobulins were normal for age, and titers to S. pneumoniae, diphtheria and tetanus were protective. Lymphocyte enumeration revealed elevated T and NK cell numbers for age. Lymphocyte proliferation to PHA, PWM, Candida and tetanus were normal. Dihydrorhodamine assay was normal. B cell phenotyping was normal. There was normal expression of CD69, HLA-DR and CD25 on activated T cells; of note the patient was on systemic steroids when tested. Invitae 207 gene PIDD panel revealed a variant in CTLA4: c.326G>A (p.Gly109Glu) that has been shown to be pathogenic in one patient (Schawb et al). Flow cytometry showed normal frequency of T follicular helper cells and T regulatory cells compared with controls, however CTLA-4 expression by T regulatory cells was lower than control. Due to the severe and progressive nature of the patient's lung disease, therapy with 3x weekly azithromycin and Abatacept 50mg SQ weekly was initiated.

We report a case of CTLA-4 haploinsufficiency presenting with recurrent pneumonia and bronchiolitis obliterans in a 2-year-old child. Based on patient registry data, our patient appears to be the youngest child diagnosed with CTLA-4 haploinsufficiency reported in the literature to date (Schawb et al). Notably, our patient lacks other features commonly described in CTLA-4 haploinsufficiency, including autoimmune cytopenias, gastrointestinal disease, lymphoproliferation, and hypogammaglobulinemia. This case illustrates the importance of consideration of this diagnosis in young children with severe lung disease without other evidence of immune dysregulation. Our hope is that prompt recognition and early treatment administration will prevent disease progression and further decrease in pulmonary function.



(42) Submission ID#803769

Looking Through The Kaleidoscope Of GATA2 Haploinsufficiency: A Novel Case Presenting With Cytopenias, Splenomegaly, Leukoencephalomyelopathy, Granulomatous Uveitis, And Recurrent Fungal Infections.

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Abstract/Case Report Text

Background:

GATA 2 is a zinc finger transcription factor essential for development, hematopoiesis, and lymphatic angiogenesis. Heterozygous mutations cause haploinsufficiency due to protein dysfunction or reduced transcription. Clinical phenotypes of GATA2 deficiency are highly variable; common phenotypes include MonoMac, dendritic cell, monocyte, B and NK lymphoid (DCML) deficiency, familial AML, and Emberger syndrome. We report a new variable presentation of the DCML phenotype.

Case: A 55-year-old male with a history of warts as a child was in good health until age 32, when incidental bloodwork revealed severe thrombocytopenia. Bone marrow biopsy demonstrated sea-blue histiocytosis with negative work-up for Niemann-Pick Disease. He underwent splenectomy with pathology showing EBV-associated lymphoid hyperplasia. Despite vaccinations prior to

splenectomy, he had an episode of pneumococcal meningitis at age 35, and sepsis of unknown origin at age 55. Over the past several years he has developed chronic tinea corporis, onychomycosis, and otitis externa infections despite numerous antimicrobial regimens.

At age 47, the patient developed urinary retention, walking, and balance difficulties. He was found to have diffuse white matter changes on MRI, elevated WBC, and positive oligoclonal bands. Initially, he was diagnosed as progressive MS treated with steroids with partial improvement. CSF microbiology studies including AFB stains, bacterial, fungal, mycobacterial cultures, cryptococcal antigen, VDRL, T. pallidum particle agglutination (TPPA), as well as, PCR for CMV, EBV, VZV, Enterovirus, HSV 1-2, JC virus and T. pallidum were all negative. Peripheral blood studies included mycobacterial blood culture, PCR for CMV, EBV, HHV-6, in addition to serology for cryptococcal antigen, and Coccidioides species, all of which were negative. Additional neurological complications include granulomatous uveitis and oscillopsia, which he developed around age 47.

Immune evaluation performed at age 51 revealed low IgG2 and IgM, and the patient was started on 30 grams of monthly IVIG. CBC with differential was notable for normal monocyte count and thrombocytopenia, mild neutropenia (Table1). Immunophenotyping revealed absent B cells and NK cells, while the CD8 T cells were elevated. CD4 T cells were normal (Table 1).

At age 55, whole-exome sequencing identified a heterozygous missense mutation in GATA2 c.1186C>T, p.(Arg396Trp). After the diagnosis of GATA2 haploinsufficiency, he was found to have Myelodysplastic Syndrome with multilineage dysplasia (MDS-MLD) on bone marrow biopsy. He is currently awaiting bone marrow transplant

Discussion: We present a 55-year-old male with cytopenias, splenomegaly, leukoencephalomyelopathy, granulomatous uveitis, and recurrent fungal infections found to have a pathogenic heterozygous missense mutation in GATA 2. Leukoencephalomyelopathy in GATA 2 haploinsufficiency has been associated with JC virus and EBV infection. Our patient did not have any evidence of a chronic CSF infection. To our knowledge, myelopathies have not been reported with GATA2 c.1186C>T, p.(Arg396Trp). This case highlights the variable nature of presentation in GATA 2 haploinsufficiency, and the need for clinical awareness of this entity in order to facilitate early diagnosis and appropriate therapy.

Complete Blood Count	Flow cytometry	Immunoglobulins*
White blood cells 4.3 x10*9 /L (N: 4-11)	**CD3 2.40 x10*9 /L (N: 0.65-2.09)**	**IgG 17.20 g/L (7-16)**
Hemoglobin 141 g/L (N: 135-170)	CD4 0.48x10*9 /L (N: 0.41-1.33)	IgA 0.92 g/L (0.7-4)
Platelets 117 x10*9 /L (N:150-400)	**CD8 1.85 x10*9 /L (N: 0.20-0.78)**	**IgM <0.20 g/L (0.4-2.30)**
Neutrophils 1.4 x10*9 /L (N: 2-8)	**CD56 <0.01 x10*9 /L (N: 0.08-0.75)**	
Monocytes 0.4x10*9 /L (N: 0.2-1)	**CD19 <0.01 x10*9 /L (N: 0.07-0.50)**	

Table 1. Selected immunological work up results. *Vaccine titres unavailable as patient is on IVIG.

(44) Submission ID#804168

Central Nervous System Lesions and later Liver Lesions in a 20-year-old male with PASLI Immunodeficiency

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Abstract/Case Report Text

Background: In 2013 the PASLI disease (p110 δ activating mutation causing senescent T cells, lymphadenopathy, and immunodeficiency) also known as Activated phosphoinositide 3-kinase (PI3K) delta syndrome (APDS) was discovered as a combined immunodeficiency.

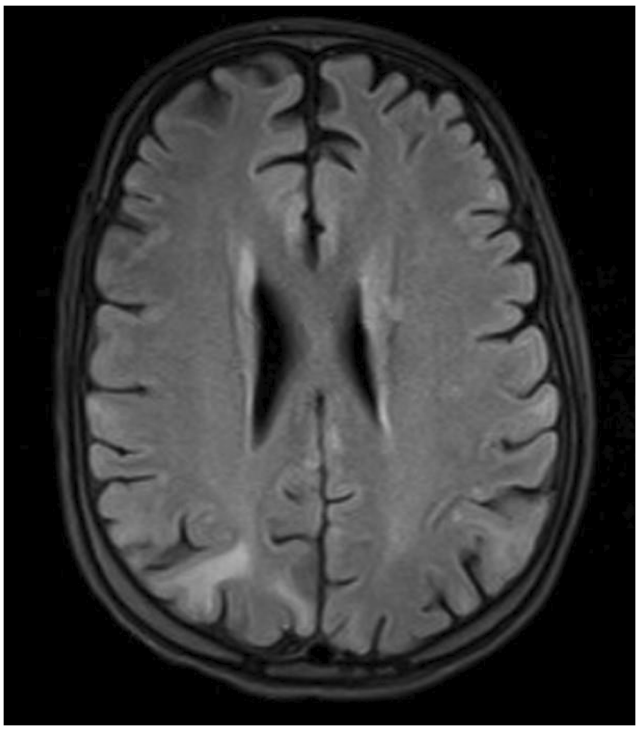
Case Description: A 20 y.o. male with panhypopituitarism, history of autoimmune hemolytic with splenectomy, and PASLI (PIK3CD or APDS1) immunodeficiency presented to our Emergency Department with a three-week complaint of right leg weakness. He also had three - weeks of constipation and urinary retention.

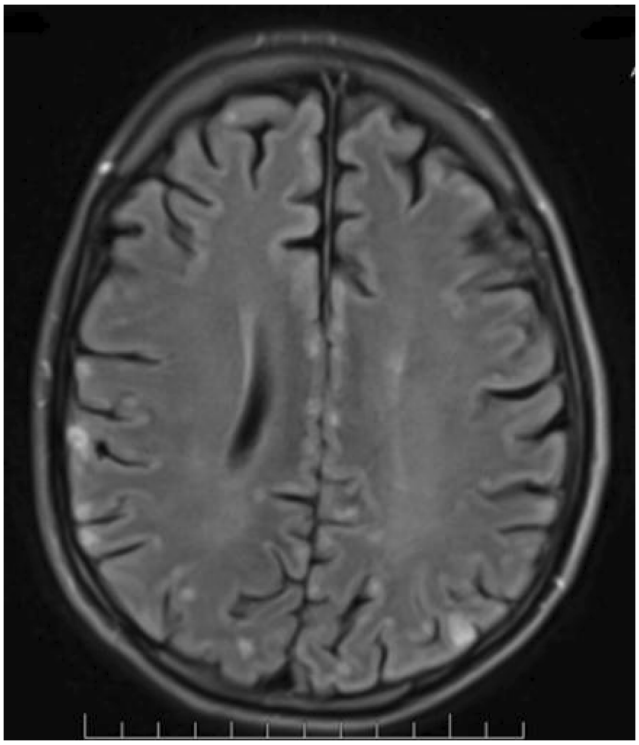
Magnetic Resonance Imaging (MRI) of the brain and spine showed numerous enhancing parenchymal nodules (figure one). Brain or spinal biopsy was requested, but not recommended by our Neurosurgery service. Lumbar puncture evaluation was performed. Cerebral spinal fluid showed no bacterial or fungal elements. Both quantiferon gold for tuberculosis and three consecutive sputum cultures for acid fast bacilli were negative. He continued his Sirolimus and the intravenous immunoglobulin replacement was increased to two grams/kg. The patient was cleared from respiratory isolation and discharged after two weeks in our facility with mild improvement in his neurologic status.

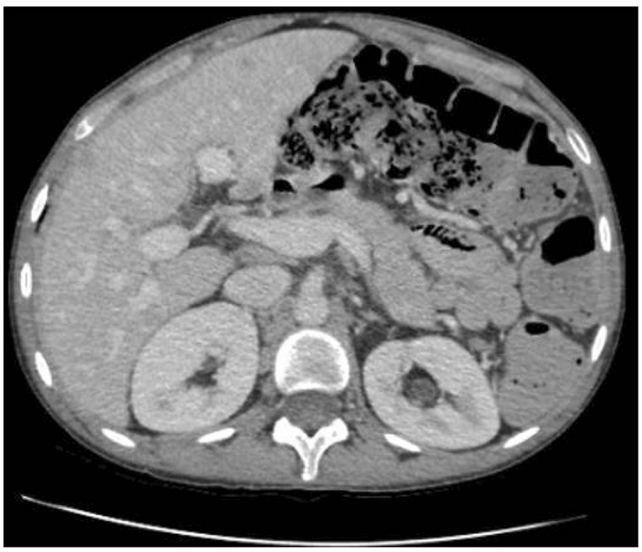
Within five days he was sent to a nationally renowned hospital. At this facility, extensive evaluation for his neurologic deficits were performed including culture and PCR for bacteria, virus, mycobacteria from CSF bone marrow, lymph node, blood, and induced sputum. These were noncontributory. He was given high dose corticosteroids for two days. One of our facility's sputum cultures was reported with acid fast bacilli. But sputum mycobacterium tuberculosis PCR was negative. Repeat MRI scans of the brain and spine showed improvement (figure 2), so no brain biopsy was performed.

He was sent back to our facility with the recommendation to start a targeted PI3Kinase inhibitor on compassionate grounds as he was not eligible for the clinical trial because his weight was less than 45 kg. But pretreatment abdominal CT revealed multiple low-density lesions scattered throughout the liver (figure 3) not previously seen on prior noncontrast CT four months prior.

Discussion: Although this gain in function mutation of the PI3Kδ signaling pathway disorder has been well characterized, this is a rare report of a patient with PASLI immunodeficiency with central nervous system and later liver lesions







(45) Submission ID#804175

MALT Lymphoma in a Patient with Autoimmune Lymphopro liferative Syndrome

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Abstract/Case Report Text

Background: Autoimmune Lymphoproliferative Syndrome (ALPS) is a rare heritable disorder of lymphocyte apoptosis. The majority of patients with ALPS have mutations in the tumor necrosis receptor family 6 gene (FAS) and these mutations are typically located within the intracellular death domain. Less frequently, FAS mutations outside of the death domain result in reduced lymphocyte apoptosis and ALPS phenotype. Mucosa-Associated Lymphoid Tissue (MALT) Lymphoma is a B-cell neoplasm, a type of indolent non-Hodgkin lymphoma. While patients with ALPS are recognized to have increased risk of various lymphomas, to date, no association has been reported in the literature with ALPS and MALT Lymphoma. MALT Lymphoma etiology lies in accumulation of lymphoid tissue in non-traditional sites, typically secondary to underlying inflammatory stimulus. It has been reported in patients with chronic infection or autoimmune disorders.

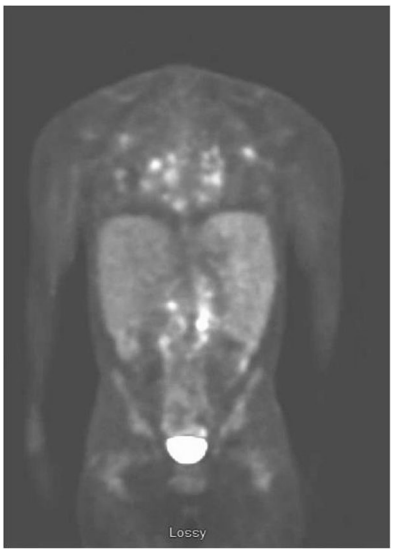
Objective: To present a novel clinical case of ALPS with associated MALT Lymphoma

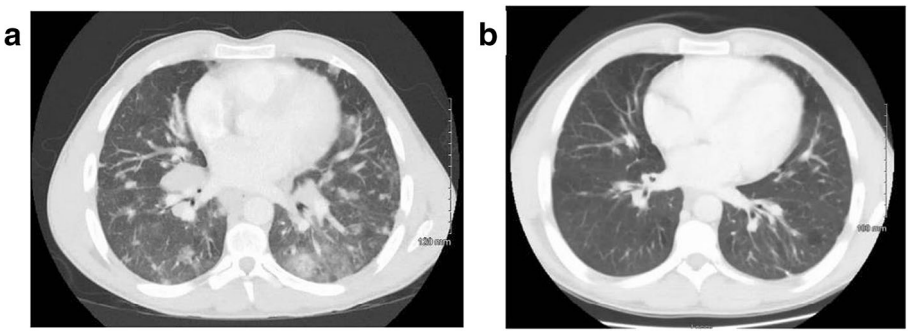
Results: The patient is a 24-year-old male diagnosed with ALPS clinically at age 8 years. He presented with inguinal and cervical lymphadenopathy, hepatosplenomegaly, and hemolytic anemia, and was found to have 10.7% double-negative T cells. He originally had increased total immunoglobulin levels, but from age 16 developed undetectable IgG and IgA, with elevated IgM. His mother had ALPS phenotype and hypogammaglobulinemia, and brother has ALPS phenotype with elevated immunoglobulins. Father and another brother are unaffected. At age 22, the patient was hospitalized following a splenic laceration in hockey. PET imaging showed subcarinal, mediastinal, retroperitoneal lymphadenopathy, splenic enlargement to 23cm and bilateral lung nodules (Figure 1). Excisional biopsies of left axillary and left lower lobe of lung were performed and showed low-grade B-cell Lymphoma (Mucosal) and underlying lymphoproliferative disease.

Invitae ALPS and CVID panels (37 genes) revealed a heterozygous variant of unknown significance in exon 3 of FAS (c.323A>G(p.Asp108Gly) Unlike most FAS mutations causing ALPS, this mutation is in the extracellular region rather than the death domain2. The c.323A>G variant has been reported in a single patient with ALPS phenotype, affecting FAS protein function by inhibiting binding to FAS ligand (FAS-L), reducing FAS-L induced apoptosis2. This FAS c.323A>G mutation was found in ALPS affected brother and was absent in the unaffected father. The patient's mother passed away prior to testing.

Since diagnosis, the patient's MALT Lymphoma has been treated with rituximab weekly for the first month and then monthly. He continues to receive monthly IVIG for hypogammaglobulinemia. After two years, CT demonstrates a significant decrease in pulmonary nodules and splenomegaly (Figure 2). The patient's Forced Vital Capacity (FVC) improved from 3.78L (67% predicted) to 4.39L (79% predicted).

Conclusion: We report the first case of MALT Lymphoma seen in a patient with ALPS. It is unknown whether the unique FAS c.323A>G mutation in the non-death domain contributes to MALT lymphoma progression. We propose MALT lymphoma is a malignant transformation of chronic inflammation that has the potential to occur in patients with ALPS. In the future, improved knowledge of mechanistic pathways of inflammation in lymphoma development and progression is important in the optimal management of ALPS.





(46) Submission ID#804395

An Evolving Immune Phenotype in a Patient with Heterozygous RAC2 Deficiency

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Abstract/Case Report Text

A Caucasian girl was born at 32 weeks gestational age due to preterm labor. The infant's initial and repeat TREC was undetectable on state newborn screen. Marked T cell lymphopenia was confirmed with CD3 57 cells/μL, CD4 40 cells/μL, CD8 13 cells/μL (naïve CD4 7.3%, naïve CD8 9.8%) and severe B and NK cell lymphopenia was also identified (CD16/CD56 83 cells/μL; CD19 12 cells/μL). Further immunologic evaluation revealed decreased mitogen proliferation to PHA (63,798; control 96,090-358,179), IgG of 350 and undetectable IgA and IgM. ADA and PNP were not decreased. The baby was started on atovaquone, fluconazole and IVIG for prophylaxis. At age 3 weeks, the baby developed an erythematous umbilical stump, erythematous ulcerative skin patches and plaques in her diaper area, and was found to have E.coli bacteremia.

Next generation sequencing of a panel of 207 primary immunodeficiency genes was done (Invitae) and identified a heterozygous variant in Rac2 (D57N), previously reported to be pathogenic with a similar clinical phenotype of severe bacterial infection, poor wound healing, umbilical stump abnormality, and severe T cell lymphopenia. Rac2 plays a critical role in neutrophil chemotaxis, rolling, adhesion, phagocytosis and superoxide production. Experimental studies have shown the D57N missense mutation a dominant negative effect on Rac2 function. This infant initially had a DHR with fMLP stimulation that was significantly impaired (6.06% fMLP ox-DHR; MFI fMLP 1.19), confirming her neutrophil dysfunction. She was switched to Bactrim for prophylaxis at 8 weeks of life. At age 10 weeks, the patient developed severe neutropenia (ANC 280 cells/μL), which has persisted to this day.

In this rare immunodeficiency, prognosis of patients with Rac2 deficiency is uncertain. While hematopoietic stem cell transplantation is the only potentially curative treatment option, little is known about the natural history of this combined immunodeficiency. This child has undergone transplant evaluation, which identified two 9/10 donors (both HLA-A mismatches), one 8/10 donor, and one potential 5/6 cord blood unit). At

the current time, parents are not interested in transplantation. The father has been identified as a possible mosaic with the D57N variant identified. He reports delayed wound healing, but otherwise clinically well. Dad has deferred laboratory immune evaluation. Now at age 1, this patient's T cell counts and function have significantly improved naturally, now with CD3 1657 cells/μL, CD4 1211cells/μL, CD8 386 cells/μ and normal lymphocyte proliferation to mitogens. Her NK cells and B cells remain markedly decreased (44 and 91 cells/μL respectively). Her ANC remains severely low (350 cells/μL). Interestingly, her neutrophil function also has improved somewhat (15.4% fMLP, MFI 1.23). She has continued on prophylaxis with IVIG, Bactrim, and fluconazole and done well clinically. She has had no further serious infections but has pronounced pathergy reactions with any minor skin trauma.

(47) Submission ID#804727

Outcomes of Hematopoietic Stem Cell Gene Therapy for Wiskott-Aldrich Syndrome

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Abstract/Case Report Text

Background Wiskott-Aldrich syndrome (WAS) is a rare X-linked disorder characterized by combined immunodeficiency, eczema, microthrombocytopenia, infections, autoimmunity and increased risk of hematological malignancies. Gene therapy (GT) using autologous CD34+ cells is an emerging alternative treatment with possible advantages over standard allogeneic hematopoietic stem cell transplant. We report the outcomes of a phase I/II clinical trial in which 5 WAS patients underwent GT using a self-inactivating lentiviral (SIN-LV) vector expressing the human WAS cDNA under the control of a 1.6kB fragment of the human WAS promoter.

Subjects and Methods: Five patients with severe WAS (clinical score 3-5) were enrolled (Table 1). CD34+ cells were transduced ex-vivo and re-infused after conditioning with busulfan and fludarabine. Two subjects (P4, P5) had autoimmunity pre-GT, manifested as skin vasculitis and autoimmune cytopenias.

Results: All subjects were alive at median follow-up of 5.1 (range 2.8-6.3) years. Multi-lineage vector gene marking was sustained over time. All had clinical improvement of eczema, infections and bleeding diathesis. WAS protein (WASP) expression was increased over baseline but remained below normal levels. Proliferation of T cells in response to anti-CD3 improved post-GT. Humoral immune deficiency improved, with normalization of IgM, and independence from Ig replacement and vaccine responses in those tested. Platelet levels increased to >50 x 103 cells/uL in only the two subjects with a VCN ≥2 in transduced stem cells. Podosome formation in monocyte-derived dendritic cells was near absent pre-GT and improved in all subjects post-GT, but only reached healthy control levels in the 2 subjects with highest VCN. In contrast to other trials using this SIN-LV, two patients (P4 and P5) had flares of autoimmunity post-GT, offering the opportunity to study the poorly understood mechanistic features of immune dysregulation in this disease. Self-reactive VH4-34-expressing B cells and CD21lo B cells remained elevated in most patients. However, despite WASP expression in FOXP3+ Tregs, those with autoimmunity had poor numerical recovery of T cells and Tregs at the time of clinical symptoms (Fig 1A). In addition, IL-10 producing regulatory B cells (Bregs) were highly deficient pre-GT, recovered in subjects who did not experience autoimmunity, but failed to recover in P4 and P5 (Fig 1B). Moreover, transitional B cells, which are enriched in Bregs and are potent inducers of Treg populations, also recovered poorly in those two subjects (Fig 1C).

There have been neither severe GT-related adverse events nor abnormal clonal expansion in transgene-marked cells to date.

Conclusion

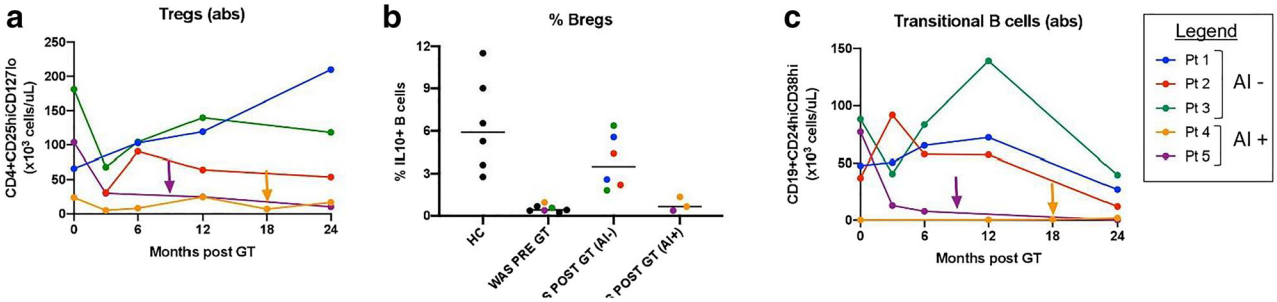
In summary, our data confirm and extend the safety and efficacy of GT in correcting disease manifestations associated with WAS, with the longest overall follow-up reported so far in studies using SIN-LV. In addition, our findings suggest that higher VCN is needed in order to correct myeloid compartments such as platelets and monocytes. Finally, we report the novel finding of the restoration of Bregs and suggest that recovery of this compartment, along with Tregs, is protective against development of autoimmunity post-GT. Overall, these data suggest a mechanism for breakdown of immune tolerance in WAS with important therapeutic implications and prognostic value.

Table 1. Patient characteristics.

Pt #	Age at GT (years)	Country of origin	Mutation	WASP+ (flow)	Revertant cells	WAS score	Platelet count ($x10^3$cells/uL)		Cell product					Bu AUC (mg*h/L)	Years F/U
							Nplate -	Nplate +	Source of CD34+ cells	CD34 + dose ($x10^6$/kg)	CD34+ bulk VCN (copies/cell)	% + CFUs	CFU VCN (mean)		
1	1.8	Turkey	Intron 3 -2A>G	↓↓↓	-	3	24-29		MPB	24.91	3.37	97%	2.35	81.2	6.3
2	3.6	USA	Exon 1 c.35del (p.Gly12fs)	Null	+ CD8+, DNT, NK	4	<10	15-20	MPB	9.34	1.34	94%	1.3	48.8	5.9
3	1.4	Japan	Exon 2 c.256C>T (p.Arg86Cys)	↓↓↓	-	3	10-15	24-66	MPB	9.8	0.54	51%	0.93	77.2	5.1
4	8	Chile	Exon 2 c.T224C (p.Trp64Arg)	Null	+ CD4+, CD8+, DNT	5	19-35		MPB	2.52	0.25	28%	1.13	84.5	5.1
									MPB	2.29	0.78	54%	1.3		
									BM	1.51	2.61	74%	2.8		
5	1.4	Vietnam	Exon 1 c.91G>A (p.Glu31Lys)	Null	-	5	12-59		MPB	15.06	1.49	69%	2.45	69.0	2.8

Abbreviations: AUC, area under the curve; Bu, busulfan; DNT, double negative T cells; F/U, follow-up; GT, gene therapy; Pt, patient; VCN, vector copy number; WASP, WAS protein.

Figure 1. A) Treg, B) Breg (2 and 5Y post-GT timepoints combined) and C) transitional B cell recovery in patients with (P4, P5) and without (P1, P2, P3) autoimmunity post-GT. Arrows represent initial time of autoimmune flares. Abbreviations: abs, absolute; AI, autoimmunity; GT, gene therapy.



(48) Submission ID#804746

Recurrent Fever and Interstitial Lung Disease

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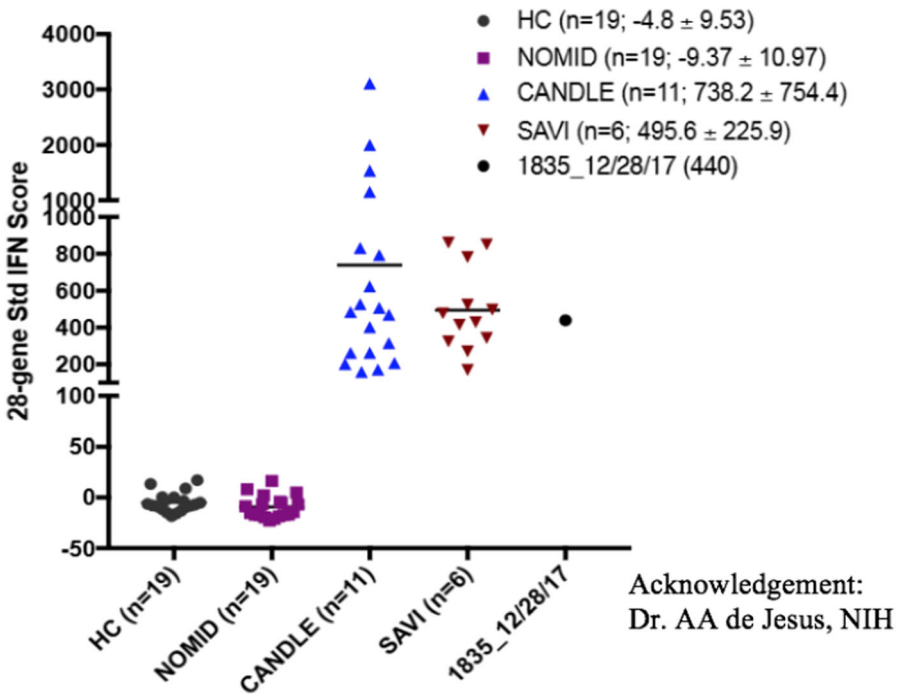
Abstract/Case Report Text

This is an 8-year-old Hispanic female who initially presented with failure to thrive, recurrent fevers and intermittent cough with episodes of perioral cyanosis. Symptoms started at age 6 months and were attributed to recurrent viral and bacterial infections. At 14 months old, she was hospitalized with fever and hypoxemia (O2 saturations 70%). CXR showed prominent interstitial lung markings and she was diagnosed with pneumonia. CT scan confirmed CXR findings and ruled out anatomical anomaly. She was lost to follow up for 2 years, and re-presented with worsening respiratory status. A repeat CT scan demonstrated worsening interstitial thickening. Immune workup, including quantitative immunoglobulins, CH50, lymphocyte subsets and vaccine response titers (pneumococcal and tetanus), was unremarkable, except for elevated IgG levels. Genetic testing for surfactant dysfunction mutations was negative. Thoracoscopic lung biopsy revealed interstitial fibrosis, PAS-positive granular alveolar proteinosis, type II cell hyperplasia, and lymphoid follicles. At age 6, she was admitted for a pericardial effusion. Rheumatology was consulted for evaluation frequent fevers and persistently elevated inflammatory markers, with concern that the pericarditis was autoinflammatory. She had an elevated ANA (>1:2560 homogeneous pattern), IL-6 (378.88 pg/mL), and IgG (2020 mg/dL) at that time. She had an atypical ANCA pattern with positive myeloperoxidase antibodies. Anti dsDNA, Smith and Scl70 were negative. She was treated with steroids and hydroxychloroquine with some improvement in her oxygen requirement. One year later she had an additional episode of pericarditis, treated with colchicine. A few months later, she was admitted with new-onset gross hematuria and elevated serum creatinine (to 2 mg/dl). Kidney biopsy showed ANCA vasculitis with glomerulonephritis (75% crescents, no scarring or fibrosis). She provisionally received a diagnosis of microscopic polyangiitis, with lung and kidney involvement. She did not have peripheral vasculopathy. She was started on cyclophosphamide, Rituximab, and IV steroid pulses. Cyclophosphamide was discontinued due to recurrent episodes of posterior reversible encephalopathy syndrome (PRES) after infusion. Her IgG level decreased as she developed nephrotic range proteinuria. A primary immunodeficiency genetic panel was sent to evaluate for monogenic immune dysregulation syndromes and revealed a TMEM173 gene mutation (c.463G>A) which has previously been reported in 4 other subjects with SAVI (STING-associated vasculopathy of infancy syndrome). STING is a cytosolic DNA sensor that leads to type I interferon production upon stimulation. This gain-of-function mutation was confirmed by measuring interferon signature gene expression at the NIH (Fig 1), and her diagnosis was revised accordingly. The patient was started on a JAK-inhibitor (Tofacitinib) to block interferon

signaling. Unfortunately, the patient is now deceased, due to overwhelming infection and multi-organ system failure. Conclusion: Genetic testing can be crucial in aiding the diagnosis of complex patients with immune dysregulation and can provide an opportunity for targeted therapy, which should be employed as soon as able to stop disease progression.



(49) Submission ID#804924

Disseminated Histoplasmosis in a Patient with PIK3CD

Amanda Holsworth, DO[1], Nicholas Hartog, MD[2]

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Abstract/Case Report Text

Background: Activated phosphoinositide 3-kinase δ syndrome (APDS-1) was first described in 2013 as a monogenetic immune dysregulation syndrome with a variable phenotype. Increased sinopulmonary and herpesvirus infections are well described, but fungal infections such as candidiasis have been rare. To date, disseminated histoplasmosis has not been described.

History: A 38 yo Caucasian male who was previously diagnosed with common variable immunodeficiency (CVID) in late childhood due to recurrent sinopulmonary infections presented with recurrent fever, pancytopenia, severe splenomegaly, and lymphadenopathy. Urine histoplasmosis antigen and beta-D-glucan were elevated. A bone marrow biopsy demonstrated granulomatous inflammation. Transbronchial biopsy of a subcarinal lymph node was consistent with granulomatous disease. This led to a diagnosis of disseminated histoplasmosis. He was treated with amphotericin B and then 11 months of itraconazole, with improvement of his symptoms. He was admitted to the hospital about 7 years later when he presented with fatigue, fever, chills, dark urine, and scleral icterus. He was found to have an acute worsening of chronic anemia with a hemoglobin of 4.7 g/dL. Due to elevated LDH, presence of schistocytes on peripheral smear, and undetectable haptoglobin, he was diagnosed with autoimmune hemolytic anemia, despite a negative direct Coombs. A bone marrow biopsy specimen was hypercellular with marked erythroid predominance, with normal flow cytometry and no blasts identified. Infectious workup was negative.

CT chest during the workup revealed new right hilar and mediastinal lymphadenopathy, in addition to calcified right hilar and subcarinal lymph nodes, bronchiectasis, and stable hepatosplenomegaly. Transbronchial biopsy of lymph nodes showed benign lymph nodes with calcified necrotizing granulomata and presence of non-viable fungal species, presumably "old" Histoplasmosis.

Family History: Family history was significant for mom dying at 29 years-old from undefined CNS infection.

Immune Labs:

· Panlymphocytopenia: Absolute lymphocyte count of 380/uL, CD3+ T cells 283/uL, CD4+ 174/uL, CD8+ 99/uL, CD19+ 65/uL, CD16+CD56+ 25/uL. CD4+/CD8+ ratio 1.8

· Decreased class-switched memory B cells and plasmablasts

· Elevated T central memory cells and activated (HLA-DR+) CD4+ and CD8+ T cells

· Hemoglobin 10.3 g/dL, platelets 77,000/uL, ANC ranging from 360/uL to 1610/uL

· IgA 107 mg/dL, IgM 273 mg/dL; reportedly had low IgG prior to initiating IVIG in childhood

· EBV PCR and CMV PCR negative

Genetics:

· PIK3CD (c.3061G>A), consistent with diagnosis of autosomal dominant APDS-1.

Discussion: Gain-of-function variants leading to increased PI3kδ activity have been shown to cause both B and T cell dysfunction, leading to impaired immunologic responses to bacterial and viral infections. Recurrent sinopulmonary infections and herpesvirus infections are commonly seen and while mucocutaneous candidiasis has been reported in cohorts of patients with PIK3CD, other fungal infections are not common. Severe disseminated histoplasmosis infections have been described in primary immunodeficiencies characterized by signaling defects in the IL-12/IFN-γ pathway, STAT3 deficiency, CD40L deficiency, GATA2 deficiency and in STAT1 gain-of-function mutations. To our knowledge, disseminated histoplasmosis has not been previously reported in patients with PIK3CD immunodeficiency.

(50) Submission ID#805384

Novel NBAS Mutation And Autoimmune Enteropathy

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Abstract/Case Report Text

The reported case represents the first case of NBAS disease detected by newborn screening program for primary immunodeficiency, based on KREC assay. The patient came to our attention due to the complete absence of KRECs and normal TRECs on DBS (dried blood spot) while hospitalized for low weight at birth (1,520 g), intolerance for enteral feeding, hepatosplenomegaly, slightly elevated liver transaminase, head and face eczematous dermatitis. During the 1st month, he also presented Klebsiella pneumoniae urinary tract infection and methicillin-resistant Staphylococcus aureus sepsis. Peculiar phenotypic features including triangular face, proptosis, flat philtrum, mild retrognatia, hirsutism, loose and slightly wrinkled skin, and apparent reduction of subcutaneous fat were noticed at birth. Complete blood count showed lymphocytopenia, marked hypereosinophilia. Serum immunoglobulin G (IgG) were markedly decreased, IgA and IgM were undetectable. Extended immune-phenotyping showed complete absence of CD19+ cells, low count of CD8+ lymphocytes, and reduced natural killer (NK) levels. At 9month of age a colonoscopy was carried out for persistent diarrhea and reduced tolerance to enteral feeding. The histological examination of mucosal intestinal biopsies showed signs compatible with autoimmune enteropathy. For this reason immunosuppressive therapy with rapamycin was started without consistent clinical amelioration. Many CVC-sepsis occurred in the last months, associated with persistent gastrointestinal symptoms and severe growth restriction. Despite the absence of experience data in literature for NBAS syndrome, we retain that HSCT represents the only resolutive therapy for him.

(52) Submission ID#805795

CADINS in an Adult with Chronic Sinusitis and Atopic Disease

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Abstract/Case Report Text

Case: A 31-year-old female with asthma and allergies presented to immunology clinic with a history of chronic fatigue and sinusitis. Fatigue occurred daily every 2-3 weeks and was described as not feeling rested even after 12 hours of sleep. Chronic sinusitis required 4-5 prolonged antibiotic courses per year. Nasal cultures grew Methicillin-sensitive and -resistant Staphylococcus aureus and Haemophilus influenzae type b (Hib). Three separate sinus surgeries over the prior few years reduced her sinus symptoms. Other infectious history was significant for recurrent urinary tract infections with E. coli and Klebsiella, recurrent otitis media as a child, and a diagnosis of transient hypogammaglobulinemia of infancy that resolved at 4 years of age.

Review of systems revealed axillary lymphadenopathy for 2-3 days twice per year not related to infection. She had longstanding eczema that responded to topical tacrolimus, multiple environmental allergies, and recently diagnosed asthma that improved with inhaled Budesonide/Formoterol. As a teenager she received allergy immunotherapy for a few years but stopped due to frequent adverse reactions. Family history revealed that father died from cancer. Physical exam was unremarkable.

Laboratory evaluation demonstrated normal IgG 874 mg/dL, IgM 133 mg/dL, IgA 166 mg/dL, elevated IgE 457 mg/dL, normal T and NK cell enumeration, mildly low total B cells (180 cells/mcl, 5.6%), and normal B cell subsets (CD19+IgM+CD27- 67%, CD19+IgM+CD27+ 17%, CD19+IgM-CD27+ 13%). Tetanus antibody titer was protective, but Hib antibody titer was undetectable at < 0.15 mcg/mL with marginal response after vaccination (0.53 mcg/mL). Pneumococcal serotype specific IgG levels (Mayo) were mostly undetectable with 3 of 23 serotypes protective at baseline and only 4 protective post vaccination with Pneumovax 23. CD3/CD28 blastogenesis was poor. Due to poor antibody response and continued sinus infections she was started on IgG replacement. Her fatigue and sinus symptoms improved moderately but she continued to require antibiotics and sinus CT scans continued to demonstrate significant disease. A focused exome sequencing panel was pursued and a novel heterozygous CARD11 variant was found (c.215G>T, p.R72L).

Discussion: The CARD11/BCL10/MALT1 (CBM) complex is a critical signaling adapter that facilitates several downstream immune responses predominately through NF-kB. Mutations in several different domains of CARD11 result in a clinical entity collectively referred to as CARD11-associated atopy with dominant interference of NF-kB signaling (CADINS). CADINS is associated with a broad range of clinical manifestations but most have marked atopy with infections, poor T cell proliferation, and varying levels of poor antibody response.

Both our variant (p.R72L) and a previously reported pathogenic variant in the same amino acid (p.R72G) involve a change from a charged arginine to a non-polar amino acid in the critical BCL10/CARD11 binding interface. IκBα degradation was not present in B cells from our patient (Figure 1) confirming the functional defect in NF- kB signaling. Thus, we present a novel variant that fits CADINS both clinically and genetically. Clinicians should be aware of CADINS when patients present with recurrent infections in the setting of significant allergic disease.

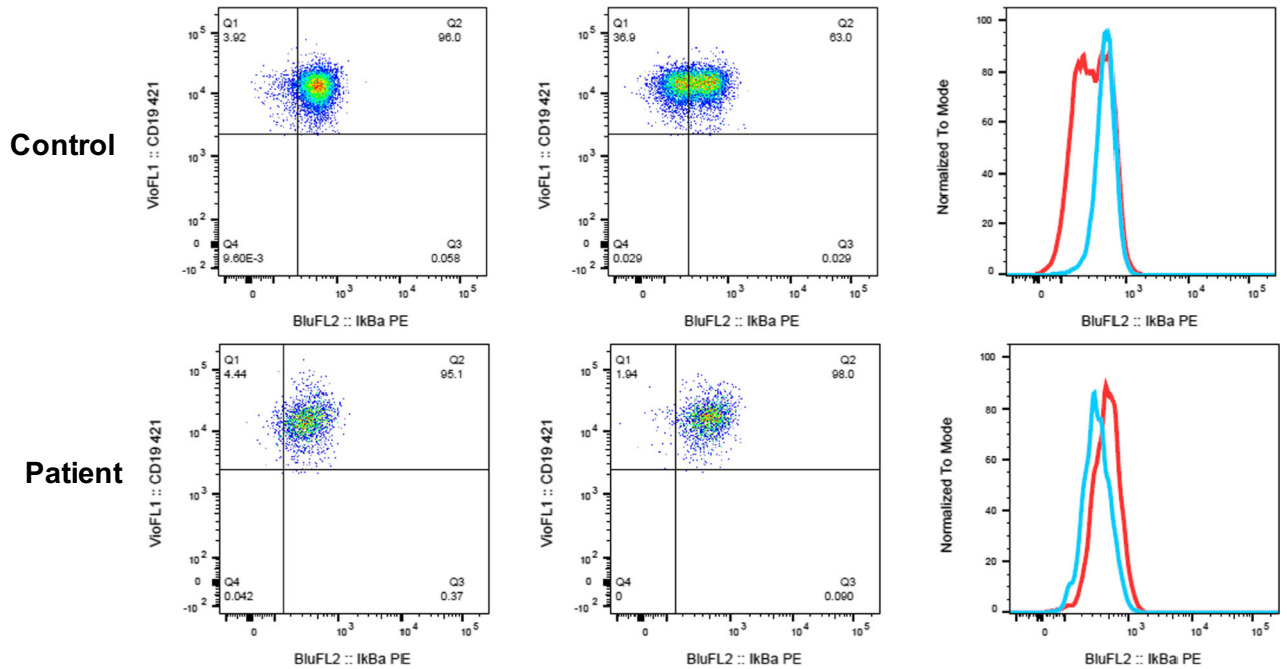

Figure 1. Defective NF-kB activation in primary B cells from patient with CARD 11 variant (p.R72L). We performed an IκBα degradation assay for the patient compared to a control. Unstimulated B cell populations are depicted in the left column. B cells were stimulated with phorbol 12-myristate 13-acetate (PMA) for 20 minutes depicted in the middle and right columns. The patient demonstrates absent IκBα degradation compared to control. (Right column: light blue line = unstimulated, red line = PMA stimulated).

I B degradation assay. *Stimulation*: 200 μL of whole blood was stimulated in a 5 mL FACS tube with 50 ng/mL phorbol 12-myristate 13-acetate (PMA; Sigma, Cat# P1585) at 37 °C for 5, 10, or 20 min, at which point 4 mL of pre-warmed 1X Lyse/Fix buffer (BD, Cat# 558049) was added. Cells were fixed for 10 min at 37 °C, centrifuged and washed twice with FACS buffer (PBS supplemented with 2% FBS and 1 mM EDTA). *Staining and permeabilization*: Fc receptors were blocked for 5 min at RT (Human TruStain FcX; Biolegend), followed by a 20 min stain on ice with anti-CD4 AF647 (clone RPA-T4; Biolegend), and anti-CD19 BV421 (clone HIB19; Biolegend). Cells were washed with FACS buffer and permeabilized for 30 min on ice with 1 mL Phosflow Perm Buffer II (BD Biosciences, Cat# 558052) that had been pre-cooled to -20 °C. After permeabilization, two mL FACS buffer was added and the samples were centrifuged. After three additional washes, the cells were stained with anti-IκBα PE (clone 25/IkBa/MAD-3; BD Biosciences) for 30 min at RT. Samples were washed three times and data were collected on a Cytek DxP10 flow cytometer. Data were analyzed with FlowJo software.

(54) Submission ID#806340

The Importance Of Considering Monogenic Immune Disorders In Adults: New Diagnosis Of Wiskott-Aldrich Syndrome In A 32-Year-Old Male

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Abstract/Case Report Text

Introduction: Wiskott-Aldrich Syndrome (WAS) is a rare, but well-defined X-linked disorder. Loss-of-function mutations in the WAS gene result in classic WAS and X-linked thrombocytopenia (XLT), while gain-of-function mutations lead to X-linked neutropenia (XLN). Classic WAS phenotypic features include recurrent infections, microthrombocytopenia and eczema along with increased susceptibility to autoimmune disorders and malignancy. Most males with classic WAS are diagnosed in early childhood and early death can result from its various clinical manifestations.

Case: We present a 32-year-old male who was referred to immunology for hypogammaglobulinemia. As an infant he had moderate eczema, and at the age of two was diagnosed with immune thrombocytopenia (ITP) with baseline platelets of 50-60 x 10^9/L. Infectious history was notable for one episode of pneumosepsis and recurrent otitis media, influenza, and herpes labialis infections. Around the age of 22, he was diagnosed with common variable immunodeficiency (CVID) based on the finding of low immunoglobulins. He developed diffuse large B cell lymphoma at age 26, and was treated with cyclophosphamide, doxorubicin, vincristine, prednisone and rituximab (CHOP-R). At age 32 he developed abdominal pain with bloody stools. Investigations confirmed an endoscopic and pathologic diagnosis of ulcerative colitis. Due to the severity of his disease, he has required maintenance therapy with vedolizumab. He was again noted to have hypogammaglobulinemia at which point he was referred to immunology at our centre. There was no significant family history of immunodeficiency, malignancy or autoimmunity. Blood work was notable for normal white blood cell and lymphocyte counts, platelets of 60 x 10^9 g/L, low IgG at 4.82 g/L (7.0-16.0 g/L) with normal IgA, IgM and IgE. Lymphocyte subsets including T, B and NK cells were within the normal range. Genetic testing was performed and he was found to have a known pathogenic mutation in the WAS gene (c.1453G>A, p.Asp485Asn) which has been previously reported in association with WAS and XLT. He has since been placed on immunoglobulin replacement and has been referred for consideration of hematopoietic stem cell transplantation.

Discussion: WAS is a rare syndrome that can have a similar phenotype to other immunodeficiency disorders including CVID, Omenn syndrome and IPEX (immune dysregulation, polyendocrinopathy, X-linked). Individuals with CVID present with hypogammaglobulinemia and recurrent infections, and these individuals also have an increased susceptibility to autoimmune disorders, gastrointestinal disease and malignancies, especially lymphoma. Although eczema is a common disorder, its presence in addition to features of early onset thrombocytopenia, immunodeficiency, autoimmunity and/or malignancy in male patients should heighten the suspicion for WAS. It is important to make the diagnosis of WAS as hematopoietic cell transplantation and gene therapy are potentially curative treatment options.

(55) Submission ID#806348

Secondary Immune Deficiencies in Hematological Malignancy: Developing International Consensus on Patient Assessment and Selection for Immunoglobulin Replacement Therapy (IgRT)

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Abstract/Case Report Text

Secondary immune deficiencies (SID) are caused by varied mechanisms and are common in patients with hematological malignancies such as chronic lymphocytic leukemia (CLL) and multiple myeloma (MM). In this setting, both the disease and its treatment (such as B cell ablation therapy) contribute to the development of secondary antibody deficiency.

Infections remain a major cause of morbidity and mortality in CLL and MM patients. This underscores the need for early recognition and stratification of risks in order to guide appropriate treatment, including immunoglobulin replacement therapy (IgRT). New guidelines for the use of human normal immune globulins in SID patients were implemented by the European Medicines Agency (EMA) in 2019. Despite these new guidelines, significant variations remain across European countries in the assessment and approaches aiming to achieve reduction in infection burden, including different strategies for initiation, dosing and discontinuation of IgRT. The same is true for North America where IgRT is widely used off-label to prevent infections in patients with SID due to hematological disease or other reasons.

In order to address this variability, a Task Force comprising both Immunologists and Hemato-Oncologists drafted 20 statements aiming to test for consensus. Statements were related to six major areas: Definition of infections, Measuring IgG levels, Initiating IgRT, IgRT dosing, SCIg usage and Discontinuing IgRT. This was followed by an international Delphi consensus exercise in three rounds which aimed to develop recommendations on how to diagnose, treat and follow-up patients with antibody deficiency associated with hematological malignancies. The first Delphi round consisted in testing the 20 statements with a panel of SID specialists and subsequently their comments were used by the Task Force to refine the statements.

In the second Delphi round, the refined statements were presented via phone interviews to the same panel to assess their level of agreement with each statement (ranging from 1 "I totally disagree" to 6 "I totally agree"). Consensus was considered to be reached per statement if 70% of the experts agreed with each statement overall.

The cut-off for overall agreement was 4 "I somewhat agree". If the expert chose level 4 or less the reasons underpinning his/her choice were discussed. Consensus was achieved for all statements on level 4 ("I somewhat agree"). Only 5 statements did not achieve consensus on level 5 "I mostly agree".

In Delphi Round 3, panelists who had not "mostly agreed" with these five statements were given the opportunity to reconsider their assessment based on the feedback from other panelists, which was shared with them. The panelists then chose to maintain or refine their assessment.

Analysis of the full results on the six key areas identified by the Task Force will be presented at the conference to offer recommendations and help guide the management of SID in patients with hematological malignancies.

(56) Submission ID#806376

Liquid versus Semi-Solid Culture Medium for Differentiation of Human Mast Cells from Hematopoietic Stem Cells in Bone Marrow

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Abstract/Case Report Text

Introduction: Mast cells (MCs) are hematopoietic-derived immune cells, whose precursors migrate within tissues reaching maturation and differentiation. Masitinib, a selective tyrosine kinase inhibitor, is efficient in controlling the survival, differentiation, and degranulation of MCs.

Aim: To optimize mast cell-differentiation from human bone marrow (BM) hematopoietic stem cells, and to find best cell culture conditions for proliferation, differentiation, and maintenance of MCs, which is important when studying particularly MCs' response to cytotoxic compounds.

Material-Methods: To produce MCs in vitro, the first method (M1) we used was a modified semi-solid culture method (1). Briefly; human BM mononuclear cells (MNCs) were obtained with Ficoll gradient from BM sample of a patient with idiopathic thrombocytopenic purpura. Colony-forming unit (CFU)-mast was developed from MNCs in methylcellulose medium supplemented with SCF (200ng/ml) + IL-6 (50ng/ml), and IL-3 (1ng/ml; only first week). 5-6 weeks later mast cell colonies were transferred into suspension cultures, in which MCs matured and multiplied up to 7-8 weeks and were used in experiments till 10th week of culture. On the other hand, in our second method (M2); MNCs were separated by Ficoll, seeded in 6 well-plates with IMDM containing FBS 2%, Pen/Strep, and a little amount of methylcellulose, and incubated at 37°C, 5%CO_2. Cultures were then supplemented with IMDM (FBS 1%) + SCF (100ng/ml) + IL-6 (50ng/ml) on day 4; and IMDM (FBS 2%) + SCF (100ng/ml) + IL-6 (50ng/ml) + IL-3 (1ng/ml) on day 9. Beginning

on day 18 till the end, IMDM (FBS 2%) + SCF (100ng/ml) + IL-6 (50ng/ml) were added to cultures.

For both methods, morphological assessment of colonies/cells were evaluated under an inverted microscope (Figure 1 and 2). Verification of MCs was performed by immunoflorescence staining for anti-tryptase and -chymase antibodies, and by toluidine blue staining. Macrophages were verified by anti-CD-68 immunoflorescence staining. MCs were exposed to masitinib or DMSO for the evaluation of dose-related effects of masitinib, and cytotoxicity was evaluated by MTT assay.

Results: In M2, culture conditions were easier to handle compared to M1. In M2, high amounts of MCs in immature and pre-mature forms were appeared as early as 15-18 days, and peak levels of proliferation rate was around 2-4 weeks of culture, which was about 3 weeks earlier than M1. Culture could be maintained till 10 weeks in both methods. Although MCs are non-adherent cells, in liquid method adherent BM cells such as fibroblasts, endothelial cells and mesenchymal stem cells have adhered to the plate and grown up, providing an attachment site for MCs and serving as a natural BM nest, mimicking in-vivo environment, for MCs to grow and proliferate (Figure 2). Attachment of MCs has provided medium exchange available without changing culture dishes. When MCs were exposed to masitinib (0.5, 1, and 2 μM/μl), approximate survival rates were 75%, 72%, 69%, respectively.

Discussion: In our liquid medium method, the adherent BM cells not only provided a natural nest supporting MC development and differentiation, they also served as an attachment site for MCs. As the cells slightly adhered, when trypsinized shortly, they easily detached and used for experiments. And we also report for the first time that adding a little amount of methylcellulose to the liquid medium provides ease of aggregation of CFUs, and easy development of MCs. We suggest that our liquid culture may be superior to semi-solid method, that it is faster and easier to handle.

Ref. 1. Ozdemir O. Evaluation of human mast cell-mediated cytotoxicity by DIOC18 target cell labeling in flow cytometry. Journal of Immunological Methods. 319, 98–103; 2007.

Figures:

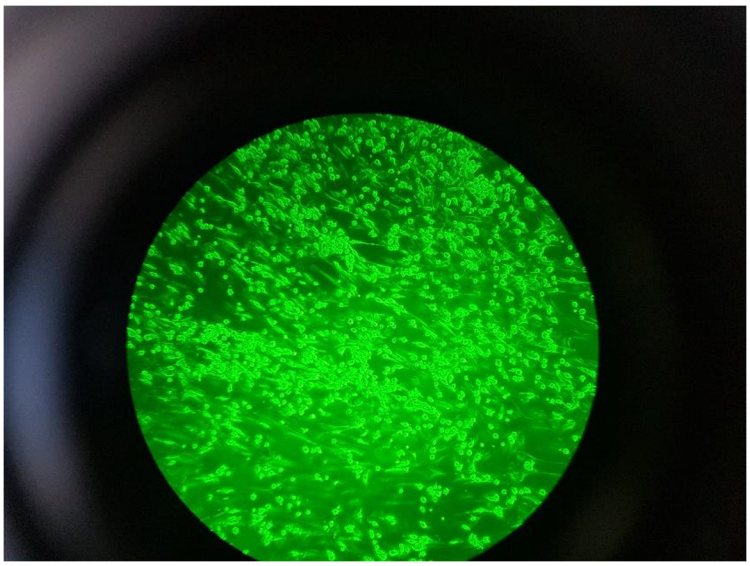

Fig. 1 Day 16. Liquid method (M2): Slightly attached massive mast cells in immature forms are seen (x50).

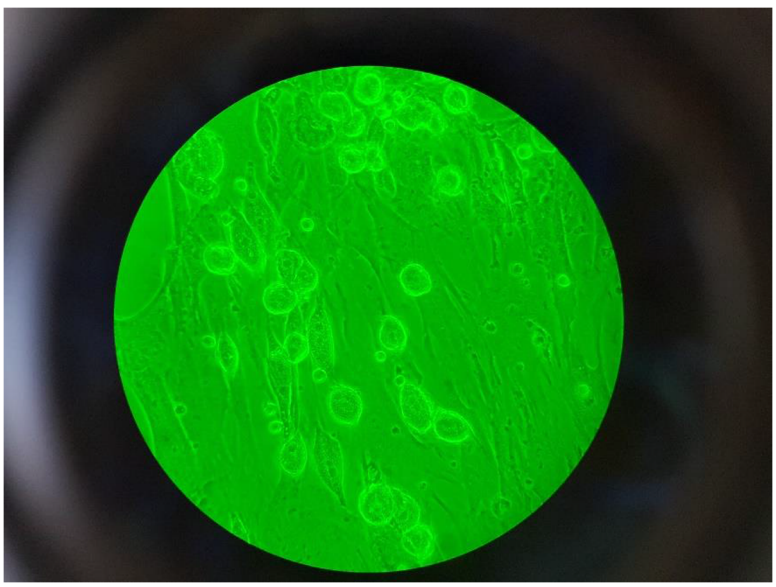

Fig. 2. Day 28. Liquid method (M2): Slightly attached mast cells in pre-mature and mature forms are seen. Some attached BM cells are also visible (x400).

(57) Submission ID#806440

Pharmacokinetic Modeling and Simulation of Subcutaneous and Intravenous IgG Dosing in Primary Immunodeficiency Patients

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Abstract/Case Report Text
A population pharmacokinetic (PopPK) analysis was conducted on data from 3 studies performed in the United States (US) and Canada. In all 3 clinical studies treatment-experienced primary immunodeficiency (PI) patients received intravenous (IV) doses of Immune Globulin Injection (Human), 10% Caprylate/Chromatography Purified (IGIV-C 10%) in the Run-in/IV Phase. In 2 studies subjects crossed over to subcutaneous (SC) IGIV-C 10%, and in the third study crossover was to Immune Globulin SC (Human), 20% Caprylate/Chromatography Purified (IGSC 20%).
A total of 95 PI patients from these 3 studies were included in the PopPK analysis and 1841 serum IgG concentrations were included in the final PK analysis. The PK of IgG following IV and SC administration was adequately described by a two-compartment model with first-order elimination from the central compartment. Administration of IGIV was modeled as an infusion directly into the central compartment. Absorption of exogenous IgG from the depot site of SC infusions into the central compartment was modeled as a first-order process with an absorption rate constant (KA). The full model was constructed by incorporation (forward selection process) of covariates of interest into the model. After completion of the covariate model development, the final model showed that IgG PK was not influenced by (a) the IGSC formulation used in the different studies (10% vs. 20%), (b) gender, and (c) age (pediatric vs. adult). Body weight was identified as a significant covariate having an effect on clearance and volume of distribution.
Based on the final PK results, serum clearance of IgG for the reference population was estimated to be 0.150 L/day. The volume of distribution of the central and peripheral compartments accounted for 3.06 L and 1.93 L, respectively. The intercompartmental clearance was 0.474 L/day, and the absorption constant from the depot (KA) was 0.246 day-1. The absolute bioavailability of IgG after SC administration was calculated as 70.5%.
The developed method was used to evaluate alternative dosing intervals following SC administration. The equivalent of a weekly IGSC maintenance dose administered 1, 2, 3, 5, or 7 times per week, or biweekly produced overlapping steady-state concentration–time profiles and similar area under the concentration versus time curve (AUC), maximum concentration (Cmax), and minimum concentration (Cmin) values. The results of the evaluation and simulations for IgG exposure following a switch from IGIV-C 10% dosing (every 3- or 4-weeks) to SC dosing further suggest that a range of dose-adjustment factors (DAF), from 1:1 to 1:1.37 would be sufficient to provide clinically effective trough IgG concentrations throughout the course of treatment at various treatment frequencies.
Current US product labeling for IGSC 20% specifies a DAF of 1:1.37 for transitioning immune globulin dosing from IV to SC, and specifies IGSC 20% dosing frequencies of weekly or more frequently (2-7 times per week).
In this PopPK analysis all SC dosing regimens evaluated theoretically would provide viable alternative administration options for maintaining adequate immunoprotection in PI patients with dosing flexibility over a range of regimens.

(58) Submission ID#806511

Canakinumab for the Management of Autoinflammatory and Multifactorial Disorders in Brazil: A Single Center Retrospective Analysis

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Abstract/Case Report Text
Background and aims: The immunological concept of autoinflammation made possible development of new cytokine target therapies and catalogue Autoinflammatory Diseases (AID). However, in 50% or more, no causative gene can be found going down to Undefined Inflammatory Syndromes (UIS). Anti-IL1 drugs (AIL1D) revolutionized some IL-1 mediated diseases, such as TRAPS, CAPS, Hyper-IgD/MKD and FMF. Nevertheless, treatment response among disorders are not the same as well as no specific study was designed for UIS. Papa et al, 2019, recently suggested the use of anakinra for the treatment of UIS, especially those refractory/intolerant to colchicine with severe or very symptomatic phenotype. This paper aims to retrospectively report for the first time the experience with canakinumab in monogenic and multifactorial disorders in a single, private center in Brazil. Patient and Methods: Patients's records that received canakinumab from January 2016 to December 2019 at Clinica Croce, IMA-Brazil, were revised. Demographic and clinical data were extracted and descriptively described. All statistical analysis are presented as: average (minimal; maximum; standard deviation). Results: A total of 23 patients with autoinflammatory diseases were enrolled and 65% (n=15) are female. Of them, 65% (n=15) patients had a monogenic disease: 26% (n=6) CAPS, 21% (n=5 FMF), 4% (n=1) MKD, 4% (n=1) homozygous NLCR4 and 4% (n=1) PAMI syndrome. Multifactorial disorders were 39% (n=9) patients : 8% (n=2) Recurrent Idiopatic Pericarditis, 8% (n=2) Schnitizler Syndrome and 21% (n=5) UIS. The average age of the first symptoms was 12,51 years (0;69;19,78) and the average age of diagnosis was 24,43 years (0;72;21,76) while the aveage of diagnosis delay was 11,59 years (1;59;15,16). All patients had used, prior to anti-IL1, corticosteroids with 100% prevalence of Cushing Syndrome and 69% (n=16) tried at least one steroid sparing agent without clinical success due to: intolerance or non-effective disease control or side effects. In the FMF group (n=5) 100% tried colchicine prior to canakinumab and this drug was not effective to 60% because of amyloidosis status and in 40% colchicine-induced hepatitis was observed. Canakinumab was effective for disease control in 75% (n=23) considering: control of clinical manifestations, amyloidosis reversion and normalization of acute reactants markers. The only side effect observed during the follow up were acute flu-like symptoms and psicomotor agitation (33,34% , n = 8). The average time of follow up is of 12,51 months (1;37;12,46). Canakinumab could be discontinued in just one patient with UIS. Conclusions: This is the first report of Canakinumab use for autoinflammatory disorders in Brazil. Canakinumab is an effective and safe drug for monogenic and multifactorial disorders control.

No serious adverse effect could be observed in the 3 years maximum follow up of this drug. Neither, no specific infectious disease more prevalent in south America, such as yellow fever, dengue, zika or chikungunya was observed.

(59) Submission ID#806681

Mediastinal Mass in Patient with Hyper-IgM Syndrome Type 2

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Abstract/Case Report Text

A 21-year-old caucasian male with autosomal recessive Hyper IgM syndrome Type 2 (HIGM2) due to AICDA mutation, diagnosed at age 1, presented with a newly developed mediastinal mass. He receives routine IVIG, pulmonary function tests (PFT's) and chest x-rays.

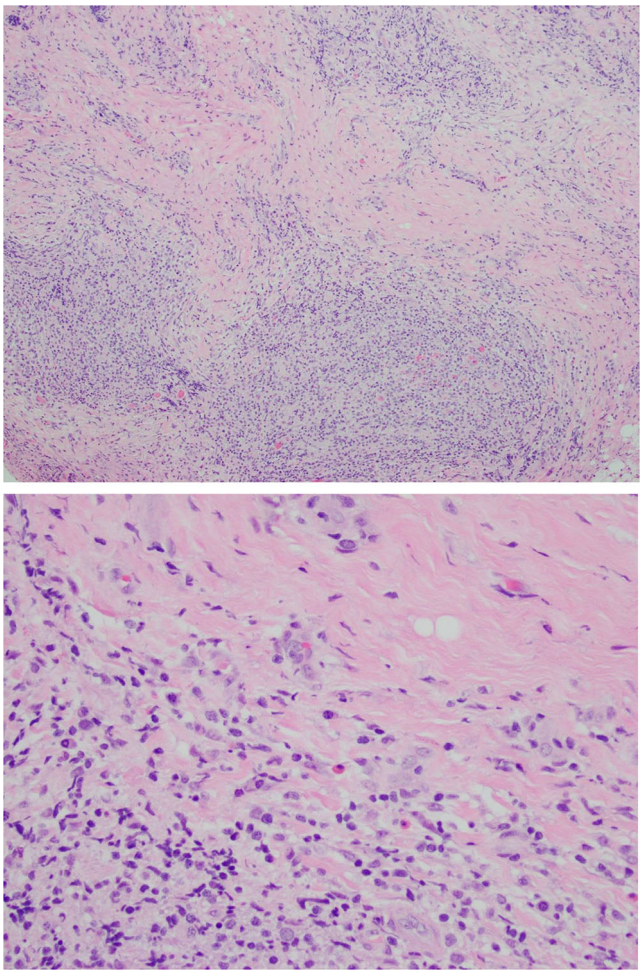
At age 16, patient was noted to have cervical and inguinal lymphadenopathy. CT scan indicated left mediastinal, hilar and pleural lymphadenopathy with soft tissue infiltration around the descending thoracic aorta and esophagus. Biopsy indicated no evidence of a lymphoma or infection.

5 years after initial workup, routine PFT's showed a declining diffusion capacity by 50%. Patient complained of intermittent chest pain but displayed no clinical symptoms of cough, dyspnea, dysphagia or reflux. CT scan which revealed an extensive ill-defined soft tissue mass extending from the thoracic outlet to the level of the esophageal hiatus that encased vascular structures resulting in narrowing and occlusion of left upper lobe pulmonary artery and left lower lobe pulmonary arteries respectively. Imaging demonstrated homogenous ventilation to bilateral lungs and decreased perfusion in the left lung compared to the right lung. Infectious workup was negative for atypical infections.

Biopsy revealed miced cellular infiltrate with no predominenant cell type or evidence of malignancy, consistent with previous lymph node biopsy 5 years prior. CD20 and CD3 stains revealed aggregates and scattered B-cells and T-cells, respectively. Removal of mass was proposed but due to the ambiguous borders and location, surgical excision was not possible.

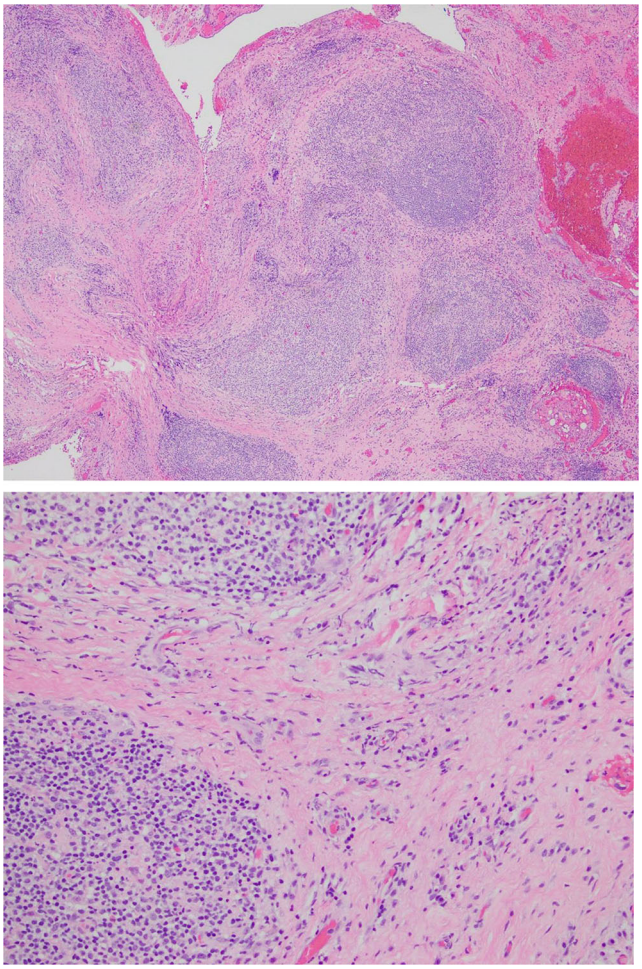
Patient was given 4 doses of Rituximab (75g), 3 doses 10mg/kg pulse steroids 18 hours apart, and daily sirolimus (level was adjusted based on sirolimus level). Follow-up CT scan indicated significant interval improvement with 50-60% reduction of the soft tissue mass. Blood flow in the left lower lobe pulmonary artery has still not returned. This may be due to collaterals and may be a separate problem from compression due to the mass.

1177 and 1175:



These two images show the mediastinal mass with extensive fibrosis and chronic lymphocytic infiltrate composed of small sized lymphocytes, plasma cells, histiocytes and occasional eosinophils

1178 and 1179:



These two images show a lymph node biopsy with surrounding fibrosis and chronic inflammation

(61) Submission ID#806742

Campylobacter species Infections as a Complication of CTLA4 Haploinsufficiency Associated Enteropathy

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Abstract/Case Report Text
Introduction:
Cytotoxic T-lymphocyte antigen 4 (CTLA-4) is an inhibitory immune regulator critical for governing T and B cell homeostasis. Heterozygous CTLA4 mutations can cause a syndrome of immune dysregulation with a variable clinical phenotype including hypogammaglobulinemia , autoimmune cytopenia and endocrinopathies, lymphoproliferation, predisposition to malignancy, tissue specific lymphocytic infiltration of brain, lung and GI tract as well as colitis.
Methods:
We retrospectively reviewed medical records of all patients with CTLA4 haploinsufficiency evaluated at the NIH between 2014-2019. A pathologic variant in CTLA4 was confirmed in all patients. We analyzed frequency of Campylobacter species detected in the stool samples by PCR based Biofilm Rapid array as well as reflex bacterial stool and blood cultures when available.
Results:
Forty-six patients aged 8-75 years were evaluated at the NIH between 2014 and 2019. Six of 46 patients (13%) had at least one episode of Campylobacter species associated acute or worsening diarrhea, with one patient also having Campylobacter bacteremia. All patients with positive Campylobacter species in stool samples had clinical histories and/or endoscopic biopsy findings consistent with enteropathy or colitis predating the incidence of Campylobacter infection. Two of the six patients (33%) had recurrent or chronic Campylobacter infection, while four of the six patients (67%) had multiple gastrointestinal pathogens detected by stool pathogen screening at various times.
Conclusions: Campylobacter species infection of the gastrointestinal tract seem to occur at an increased incidence in our CTLA4 haploinsufficient cohort. To the best of our knowledge, this is the initial report for the association between CTLA4 haploinsufficiency and Campylobacter species infection of the gastrointestinal tract. Although CTLA-4 is a critical immune checkpoint involved in mucosal immune homeostasis and gut microbiota-immune system cross talk, the underlying mechanism predisposing to Campylobacter infection in CTLA-4 deficient patients remains to be explored. Our study suggests screening of stool for Campylobacter species in patients with CTLA4 haploinsufficiency associated enteropathy.

(62) Submission ID#806771

IKAROS Dimerization Defects Associated With Hematologic Cytopenias and Malignancies

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Abstract/Case Report Text

IKAROS transcription factor and IKAROS family members are critical for development of lymphocytes and other blood cell lineages. Full length IKAROS (isoform 1) contains six C2H2 zinc fingers (ZF), four N-terminal DNA binding ZF and two C-terminal dimerization ZF. Somatic IKAROS mutations and deletions have been associated with increased predisposition to B- Acute lymphoblastic leukemia (ALL) as well with poor disease prognosis. Recently, germline IKAROS mutations affecting the N-terminal DNA binding domain and acting in a haploinsufficiency or dominant negative manner were reported to be associated with common variable immunodeficiency (CVID) and combined immunodeficiency (CID), respectively. Herein we describe a novel set of germline heterozygous IKAROS allelic variants affecting the C-terminal dimerization domains in four unrelated families. Clinical manifestations include hematopoietic cytopenias presenting as Evans syndrome, and hematologic malignancies including T-cell ALL and Burkitt lymphoma; other manifestations observed were B-cell lymphopenia and hypogammaglobinemia, but recurrent or severe infections were not prevalent or characteristic. We demonstrate that mutants affecting dimerization abolish IKAROS homodimerization as well as heterodimerization with IKAROS family members AIOLOS and HELIOS. These variants also affect DNA binding at dimerization sites and pericentromeric targeting. Opposed to previous allelic variants reported, dimerization changes alter post-translational sumoylation and gene transcription regulation. Our data show that mutations affecting IKAROS dimerization are mainly associated with cytopenias and/or malignancies, have a different mechanism of action than previously reported variants, present with incomplete clinical penetrance, and contribute to the growing spectrum of genotype-phenotype IKAROS associated diseases.

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Secondary And Incidental Findings in A Cohort of Patients with Immune-Mediated Disease Undergoing Exome Sequencing

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Abstract/Case Report Text

Introduction: There has been much discussion regarding the return of secondary findings in genetic sequencing research. Opinions differ on whether researchers should return secondary findings to participants at all and if so, what the best method is to do so. We have opted to systematically identify and return pertinent secondary findings to participants in our cohort of patients with immune-mediated diseases that undergo exome sequencing. Additionally, exome sequencing may determine multiple or other genetic diagnoses in addition to the primary diagnosis, which we call "incidental findings." Here, we discuss the secondary and incidental findings discovered in our cohort thus far.
Methods: Individuals in our protocol underwent consent for exome sequencing, including a discussion of the possibility of secondary findings. Exome sequencing data was analyzed, and variant pathogenicity was scored using the ACMG criteria (Richards et al); Variants determined to be likely pathogenic, pathogenic, or otherwise clinically important were confirmed via CLIA-certified sanger sequencing. Confirmed variants were returned to participants. We then queried internal databases for cases involving secondary and incidental findings.
Results: As of November 2019, exome sequencing, interpretation and reporting had been completed for 629 participants. We detected a total of 18 secondary findings in 17 (2.7%) participants, including variants in APOB, BRCA1(2), BRCA2 (5), DSP, FBN1, KCNH2, LDLR, MYBPC3 (2), RYR1, PKP2 (2), and VHL. Additionally, we detected possible dual/multiple genetic diagnoses in 18 (2.9%) participants, some of which explained an unusual clinical presentation or symptom. These included individuals with variants in multiple immune-related genes, including one individual with variants in GATA2 and TNFRSF1A, and those with variants in genes related to multiple organ systems, including an individual with variants in IFNGR1 and SCO2.
Discussion: Exome sequencing in this cohort detects not only important secondary findings, but also discovers a significant portion of individuals with multiple genetic diagnoses. Notably, exome sequencing may provide further context or explanation for unusual phenotypic presentation and help determine specific symptom etiology even when a primary genetic etiology is already known.

Additionally, these secondary and incidental finds may be important to consider when delineating risks and symptoms of novel or recently-discovered conditions.

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Utilizing Advanced Practice Providers In A Multidisciplinary Clinic Focused On Care Of Patients With Immune Dysregulation And Lymphoproliferative Disorders

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Abstract/Case Report Text
Background: Immune dysregulation and lymphoproliferative disorders including ALPS like disease, HLH EBV driven lymphoproliferative disease leading to rare lymphomas require a multidisciplinary approach utilizing expertise in Immunology and Hematology/Oncology to care for these patients as we learn the molecular etiology of their underlying disorders. At Texas Children's Hospital, the Immunology Lymphoproliferative evaluation and diagnostic (iLEAD) clinic was created to provide a comprehensive clinical and research approach to caring for patients with these rare disorders. In an effort to streamline care and access, we recently on-boarded an advanced practice provider (APP) .
Methods: A chart review was conducted 6 months before and after on-boarding the APP for iLEAD patient visits. We reviewed the following patient care and access parameters to determine increase in efficient and effective patient care as well as improved access to the clinic. These parameters included: Referral Process, time of referral placement to appointment, number of patient visits, wait time in clinic, lab interpretation and reporting time for disseminating results to families, and collaboration process with other specialties.
Results: Within 2 months of the APP starting our average wait from placing the referral to first appointment fell by an average of 45%. In addition, we created an algorithm to prioritize patients with immediate need to be seen. By streamlining the referral process and patient priority, we developed a "pre-clinic" conference process by which all patients are reviewed and preliminary plans are made prior to the patient's arrival. This has translated into our ability to increase the number of patients seen in clinic from 4 to 6 and decreased the wait time in clinic by approximately 30 minutes. Since the APP started, no patient has been in clinic for more than 60 minutes. This has also led to an increase in RvU generation. In terms of efficiency in patient care, all labs are now ordered while in the room with the patient by the APP and physician providers. In turn, all labs are resulted directly to the APP who reviews labs, collaborates with physicians for care and reports to families in a timely fashion within 1-2 weeks of labs being resulted compared to greater than 1 month previously. To improve collaborator communication and post visit plans, a post-visit clinic summary was created. This has been effective in reducing the time to other specialty referrals, follow up visits and effective care for ongoing clinical needs.
Conclusions: The addition of an APP in our iLEAD multidisciplinary clinic which provides specialized care for patients with immune dysregulation and lymphoproliferative disorders effectively increases work productivity of providers and enhances patient care by increasing access to care, decreasing wait time in clinic and time of reporting of results and future plans. The APP with knowledge and expertise in immunology and immune dysregulation is a cost effective way to enhance provider and patient support. With the overwhelmingly positive results, future plans include expanding our multidisciplinary clinic to other services that care for patients with suspected immune deficiency.

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Genomic Characterization of a Pediatric Cohort with Lymphoproliferative Disorders

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Abstract/Case Report Text

Introduction: Pediatric lymphoproliferative disorders represent a clinically and genetically heterogeneous group of conditions. Misdiagnosis and delayed diagnosis can contribute to substantial morbidity and mortality. Identification of molecular etiologies and underlying disease mechanisms may facilitate timely interventions and guide targeted or curative therapies.

Methods: The study was performed through retrospective chart reviews in accordance with all local ethics and IRB committees. The study was designed to investigate a cohort of pediatric patients who met criteria for non-malignant lymphoproliferative disorders from Texas Children's Hospital and collaborating centers for underlying genetic etiologies.

Results: A total of 51 affected individuals from 47 families met criteria. Distribution between male and females was nearly equivalent: males (n = 26) and females (n = 25). Approximately half of the cohort was Hispanic (n = 25). Overall Kaplan Meier survival was 67% (n = 39). Whole exome sequencing was performed in all subjects and available family members. Likely disease-causing genetic defects were identified in 29 of 47 families (62%). Within these 29 families, 20 (69%) carried variants in genes in International Union of Immunological Societies established primary immunodeficiency diseases. Potential novel genetic causes of immune deficiency or immune dysregulation were also discovered. Mechanistically, all of the implicated genes had roles in modulating lymphocyte activity; initial activation, cytoskeletal organization, or apoptosis of lymphocytes; or regulation of inflammation. All subjects less than one year of age had an identified gene in one of the three mechanistic categories with the dominant mechanistic genetic category being defective control of lymphocyte signaling (57%). In addition, 72% of patients between 1 and 8 years of age were found to have a potential genetic diagnosis underlying the LPD, with a more equal distribution of mechanistic categories compared to patients greater than 15 years of age where only 33% have a genetic cause. Other important disease manifestations identified were EBV-associated disease in 21 subjects (41%) and 15 subjects (24%) met HLH-2004 criteria.

Conclusion: Primary immunodeficiency diseases and other genetic abnormalities of the immune system underlie a significant percentage of pediatric lymphoproliferative disorder cases. Greater than 72% of patients less than 8 years of age have a genetic etiology underlying the lymphoproliferative disorder. Many of these gene defects can be treated with targeted therapies or hematopoietic stem cell transplantation. Genetic testing therefore plays an essential role in the diagnosis and management of children with these conditions.

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More Than Meets the IPEX (Immune dysregulation polyendocrinopathy enteropathy X-linked syndrome): Finding the Right Source in an Immunosuppressed Patient

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Abstract/Case Report Text

A 21-year-old gentleman with a history of immune dysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome with known pathogenic variant in FOXP3 presented to our emergency department with two witnessed episodes of tonic-clonic seizures earlier that day. He has had a longstanding history of recurrent infections and autoimmune conditions since birth, and was being treated with monthly IVIG infusions and sirolimus while awaiting bone marrow transplantation. His symptoms on admission included foaming at the mouth, generalized shaking, bladder incontinence, and tongue biting that lasted about five minutes. Family reported recent sores inside his mouth and lips, but denied any recent fevers, neck pain, headaches, chest pain, abdominal pain, nausea, vomiting, and sick contacts. He lives on a farm with livestock and reportedly had recent tick exposure. His last IVIG infusion was two weeks prior to admission, at which time he also received inactivated flu vaccine.

In the ED, a third seizure was witnessed by multiple medical providers. He subsequently received lorazepam and lacosamide with interval improvement. He underwent diagnostic lumbar puncture, as well as extensive evaluation for infections. He was started on empiric antibacterial and antiviral meningitis coverage. Analysis of the CSF showed a lymphocytic pleocytosis; bacterial cultures and HSV 1/2 PCR were negative, as was a 14-pathogen meningitis/encephalitis panel performed by PCR. EEG was negative for seizure-like activity, and brain MRI showed mild atrophy without sclerosis in the left hippocampus. Subsequently, anti-infectious therapy was stopped, and patient was discharged with outpatient follow-up scheduled for suspected non-infectious aseptic meningitis that was potentially triggered by flu vaccination versus IVIG. On day four post-discharge, however, PCR for Ehrlichia chaffeensis in the serum returned positive, and he was started on oral doxycycline.

Ehrlichiosis is a rare tick-borne illness that may cause various non-specific symptoms including fever, headaches, myalgias, and generalized malaise. Most prevalent in the mid-Atlantic regions of the United States, tick-borne Ehrlichia spreads through the mononuclear phagocytic system and can infiltrate many organs including the kidney, liver, lungs, and heart. CSF penetration can cause sometimes fatal meningoencephalitis. Aseptic meningitis due to Ehrlichiosis has been described in recent literature. Cases in HIV patients and transplant patients on chronic immunosuppressive therapy have been severe, resulting in organ dysfunction in many instances and death in a few. However, this case marks the first documented Ehrlichia infection in a patient with primary immunodeficiency. This patient's presentation of aseptic meningitis and clear exposure history fits the clinical picture. His relatively benign course could be due to preserved T effector function not seen in persons with HIV or transplant patients with significant immunosuppression.

Patients with IPEX usually present with autoimmunity and allergies, but are also prone to significant infections. It is important to perform a comprehensive workup, including testing for atypical infections, in patients with immune dysregulation syndromes who present with symptoms of unclear etiology. Special attention should be paid to patients who live in areas with known endemic exposure risks. Empiric antibiotic therapy may need to be considered early to prevent delays in treatment.

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PGM3 Deficiency: A New Spectrum of Phenotypes

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Abstract/Case Report Text
Introduction
Autosomal recessive hypomorphic mutations in PGM3 have been described to result most commonly in either hyper-IgE or severe combined immunodeficiency (SCID) clinical phenotypes in humans, with one report of an individual with combined immunodeficiency without atopy. Herein, we describe a series of individuals newly diagnosed with PGM3 deficiency functionally confirmed using lectin-based flow cytometric analysis of peripheral blood mononuclear cells, that broadens the associated clinical phenotypes to confirm CID without atopy and childhood Evans syndrome. In addition, we present 3 new disease-causing PGM3 variants, and functionally confirm the pathogenicity of a fourth (p.I350T).
Classical HIES Phenotype
Cases 1.1 and 1.2 identify 2 sisters of Spanish descent with a classical hyper-IgE phenotype. The younger sibling demonstrated severe atopic dermatitis, mild-moderate asthma, multiple food allergies, one episode of ITP, and ADHD. The older sibling demonstrated atopic dermatitis, skin infections, and C. albicans otomastoiditis. The siblings were found to have the damaging compound heterozygous variants p.T492I and p.Q506X in PGM3. Case 2 is a 15 year-old Guatemalan boy with prominent atopy including asthma, allergic rhinitis, food allergy, elevated IgE, atopic dermatitis, as well as oral HSV who was found to be homozygous for the damaging PGM3 variant p.I350T.
CID phenotype with a paucity of Atopy
Case 3 is a 10-year-old Turkish girl who is the daughter of a consanguineous union. She presented with infantile nephrotic syndrome at 6 months of age, and subsequently developed leukopenia, neutropenia, and low IgG. Complications include bronchiectasis, sinusitis, Pseudomonas urinary tract infection, and inflammatory skin lesions without atopy. She was found to be homozygous for the damaging PGM3 variant p.R69H
Evans syndrome
Case 4 is a 5-year-old girl from Guatemala. She developed multilineage autoimmune cytopenias including immune thrombocytopenic purpura (ITP), autoimmune hemolytic anemia (AIHA) and autoimmune neutropenia (AIN) at the age of 2 years, refractory to multiple treatments and finally responding to mycophenylate mofetil. She has a history of mild

eczema but is without other atopy and suffered from multiple invasive bacterial infections. An additional patient, case 5, was diagnosed with Coombs positive AIHA and ITP at age 3 years refractory to multiple treatments and finally responsive to cyclosporine. Cytopenias recurred 1 year later, resulting in hypoxic brain injury. He died of infectious complications at the age of 7 years. Both patients were found to be homozygous for the damaging PGM3 variant p.I350T.

Discussion This is the first report of PGM3 deficient individuals presenting with Evans syndrome as a primary presentation without additional pathology. While disease-associated mutations appear to cluster around the 4 key conserved domains of the protein, no clear genotype-phenotype correlation is readily observed. In addition to autoimmune cytopenias, PGM3 deficient individuals have also been reported with splenomegaly, lymphoma, and EBV viremia. Thus, in particular for children with lymphoproliferative disease, PGM3 deficiency should also be considered in the differential diagnosis.

Table 1. Patients identified with validated PGM3 disease-associated mutations.

	Case 1.1	Case 1.2	Case 2	Case 3	Case 4	Case 5
PGM3 variants (NM_001199917.1)	p.T492I, p.Q506X	p.T492I, p.Q506X	p.I350T, p.I350T	p.R69H, p.R69H	p.I350T, p.I350T	p.I350T, p.I350T
Age (years)	20	8	12	11	5	7
L-PHA on naïve CD4+	Low	Low	Low	Low	Low	*
Country of origin	Spain	Spain	Guatemala	Turkey	Guatemala	Mexico
Allergy	Eczema IgE 23,969	AD, food, asthma IgE 4,652	AD, AR, food, asthma IgE 16,172	None IgE 55-436	Eczema IgE 12	None IgE 77.7
Infections	Skin superinfections; otomastoiditis (*C. albicans*) onychomycosis;	None	Skin abscesses; necrotizing pneumonia (*S. aureus*); pericarditis; oral HSV	Pneumonia, sinusitis, rotavirus gastroenteritis; bronchiectasis	Skin abscesses (*S. aureus, Strep sp.*); pneumonia; bacteremia; recurrent acute otitis media	MRSA cellulitis, norovirus, pneumatosis intestinalis, Klebsiella and enterococcus sepsis, Varicella
Neutropenia	None	None	Episodic	Episodic	Episodic	Episodic then persistent
Lymphopenia	Low total and naïve CD4	Low total and naïve CD4	Low CD4+ and CD19+	Low total, CD4 and CD19		Pan-lymphopenic
Humoral abnormalities	None	None	Elevated IgA	Low IgG, Elevated IgA, Normal IgM	Elevated IgA, IgG, IgM	Poor vaccination response
Cytopenias	None	ITP	Neutropenia	Neutropenia, ITP	ITP, AIHA, AIN	ITP, AIHA, AIN
Bone marrow	Not done	Not done	None	hypocellular	Normocellular	Normocellular
Splenomegaly	None	None	None	None	None	Present
Neurocognitive abnormalities	None	ADHD	Seizure	Impaired speech fluidity; seizures; hearing loss		Seizures due to hypoxic brain injury

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Invasive Cryptococcal and Moraxella Infections in a Patient with Idiopathic CD4+ T Cell Lymphopenia

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Abstract/Case Report Text

Introduction: Meningitis is a life-threatening manifestation of Cryptococcus neoformans (C. neoformans). It occurs in increased frequency in those with impaired cell-mediated immunity, especially those with HIV/AIDS. Infection with C. neoformans has been seen in previously healthy individuals diagnosed with idiopathic CD4 lymphopenia (ICL). ICL is defined by an absolute CD4+ count of less than 300 cells/m3 on multiple occasions, usually 2 to 3 months apart, without other apparent cause such as HIV infection, immunodeficiency, or immunosuppressive medications.

Case Description: Our patient is a previously healthy 50-year-old female with Cryptococcus meningitis and fungemia. Her course was complicated by elevated intracranial pressure requiring extraventricular drain. She was treated with amphotericin and flucytosine for 1 month. Notably, the patient was also found to have Moraxella catarrhalis (M. catarrhalis) bacteremia without identifiable source. She denied history of environmental risk factors, was not up to date on cancer screening, and recently returned from a trip to Italy. Initial evaluation revealed lymphopenia (422 cells/uL), low CD3+ (283 cells/uL), CD4+ cells (42 cells/uL), and CD16/56+ (31 cells/uL), but normal CD8+ (234 cells/uL) and CD19+ cells (121 cells/uL). HIV, ANA, leukemia/lymphoma flow cytometry panel was negative. She also had a normal lymphocyte proliferative responses to PHA (66.7%),

normal CD45RA:RO, and protective tetanus titers (2.31 IU/mL), but only 1/23 protective pneumococcal serotypes. Initial immunoglobulins demonstrated slightly low IgG (616 mg/dL). Laboratory studies 2 months after presentation demonstrated improved lymphopenia (800 cells/uL) continued low CD4+ cells (86 cells/uL), but normalized IgG levels (645 mg/dL). Follow-up labs also demonstrated decreased CD19+ B cells (44 cells/uL) and insufficient response to polysaccharide vaccine (9/23 pneumococcal serotypes). Three months after discharge, she is continued on daily fluconazole without recurrence of infections although she still has diplopia and headache.
Discussion: In a review of cryptococcosis in 53 patients with ICL, 7 of them had cryptococcal infection in both the CNS and blood. Of these 7 patients, 1 was cured, 2 improved, 3 relapsed and then improved, and 1 died. Three of these patients were treated with amphotericin and flucytosine. Five of these patients had underlying disease and 3 had notable infections with VZV, TB, or HPV, however other infections such as M. catarrhalis were not mentioned. M. catarrhalis bacteremia has been described in children with underlying immune dysfunction and respiratory infection as well as secondary to pneumonia with M. catarrhalis. In 24 cases of M. catarrhalis bacteremia in adults, most had underlying malignancy and/or neutropenia, predisposing respiratory factors, or source for infection.
Conclusion: This report of C. neoformans meningitis and M. catarrhalis bacteremia in the setting of ICL is unusual in that to our knowledge, M. catarrhalis bacteremia has not been reported in ICL. Cases like this also raise the question as to whether some laboratory abnormalities are secondary to infection, treatment, or underlying disease. It is important to report these cases with ICL in order to group disease phenotypes, as continued monitoring and data collection of these cases may lead to discovery of new disease processes.

(70) Submission ID#806998

Regulation Of Humoral Immunity By Cytokine-Mediated STAT Signalling - Lessons From Inborn Errors Of Immunity

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Abstract/Case Report Text
Cytokines play critical roles in regulating the development, survival, differentiation and effector function of immune cells. Cytokines exert their function by binding specific receptors on the surface of immune cells and typically activating intracellular JAK/STAT signaling pathways, resulting in induction of specific transcription factors and regulated expression of target genes. In order to differentiate into an appropriate effector fate, lymphocytes need to integrate multiple signals often provided concomitantly by numerous cytokines that activate shared transcription factors. How these signals are balanced and regulated to yield the optimal class of immune response remains to be completely determined. Inborn errors of immunity, or primary immunodeficiencies (PIDs), result from germline mutations in defined genes, leading to loss-of expression, loss-of function, or gain-of function of the encoded protein. PIDs are characterised by defects in immune cell development, or their differentiation into effector cells during immune responses, thereby rendering patients not only highly susceptible to infectious diseases, but also autoimmunity, autoinflammation, allergy and cancer. PIDs are thus an unprecedented model to link defined monogenic defects to immune dysregulation in clinical settings. Indeed, PIDs have unequivocally revealed non-redundant roles of single genes, molecules, signaling pathways and lymphocyte subsets in host defense and immune regulation, and formed the basis of better therapies for immunopathologies. Our indepth analysis of inborn errors of immunity of cytokine signalling pathways have identified fundamental requirements for generating long-lived humoral immune responses in humans. Here, I will present data relating to our recent studies of how inactivating mutations in IL21R, IL6R, ZNF341, STAT1, STAT3, and STAT5, disrupt or dysregulate the generation and function of human memory B cells and Tfh cells, thereby precipitating humoral immunity, as well as allergic disease and autoimmunity.

(72) Submission ID#807071

Immunophenotyping of cytologic Specimens by Flow Cytometry in Patients With or Without Prior Hematologic Malignancy

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Abstract/Case Report Text
Introduction: Flow cytometry is a powerful diagnostic tool for detecting hematologic malignancies in a variety of patient specimens including body fluids and lymph node aspirates. Cytopathologists are frequently confronted with lymphocyte – rich effusions, and the definite decision of whether the lymphocytosis is of a purely reactive nature or a presentation of an indolent lymphoma may be an extremely difficult based on microscopy alone. Moreover, small proportions of malignant cells that may be missed out by routine morphology can be detected by flow cytometry.
Objective: The purpose of this study was to evaluate the usefulness of multiparametric flow cytometry Immunophenotyping (FCI) to confirm the presence of leukemia or lymphoma cells in body fluids and FNA specimens.
Methods: Body fluids and FNA specimens simultaneously obtained for FCI, cytologic analysis and Real time PCR from 30 patients were submitted to our flow cytometry laboratory from January 2017 to September 2019. The samples studied were 16 body fluids (11 pleural fluids and 5 ascitic fluids) and 14 FNA samples (13 enlarged lymph nodes and 1 lung mass).Four color FCI method was performed and the following fluorescent monoclonal antibodies were used: CD45, CD19, CD5, CD20, CD22, CD23, CD79b, FMC7, Kappa and Lambda light chains, CD200, CD123, CD10, CD11c, CD2, CD1a, CD3, CD5, CD7, CD4, CD8, TdT, CD52, CD25, CD30, CD40, CD56, CD95, BCL2, CD34. FCI analysis was performed on a Beckman coulter cytomics FC500 flow cytometer using software CXP to analyze data. The cases were diagnosed as leukemia or lymphoma as per the World Health Organization (WHO) 2008 guideline. Real time PCR was done for detecting Mycobacterium tuberculosis (MTB) DNA to exclude tuberculosis.
Results: Among 30 cases 27(90%) showed immunophenotype positivity for malignancy, of which 25/27(83.33%) were hematologic malignancies and 2/27(6.67%) other malignancies. MTB DNA was positive in 3/30(10%) cases. Pleural fluid (n=11) samples were positive for diffuse large B-cell lymphoma (DLBCL) (4 cases), angioimmunoblastic T-cell lymphoma (4 cases), T-lymphoblastic lymphoma (1 case), thymoma (1case),

tuberculosis (1 case). Ascitic fluid (n=5) samples showed positivity for angioimmunoblastic T-cell lymphoma (4 cases) and DLBCL (1 case). FNA of lymph nodes (n=13) were positive forT-lymphoblastic lymphoma (2cases), angioimmunoblastic T-cell lymphoma (2 cases), DLBCL (3 cases), Hodgkin lymphoma (1case), nodular lymphocyte predominant Hodgkin lymphoma (1 case), peripheral T-cell lymphoma (NOS) (1 case), splenic B-cell marginal zone lymphoma(1 case), tuberculosis (2 cases). One FNA of lung mass were tumor of neural cell origin. Both immunophenotype and cytomorphology positive for malignancy were in 19/30(63.33%) cases.Cytomorphology was negative/ suspicious in 11/30(36.67%) cases, of which both cytomorphology and immunophenotype negative were 3(10%) and cytomorphology negative but immunophenotype positive cases were 8(26.67%). MTB DNA was detected in pleural fluid in 1 case and FNA sample in 2 cases.

Conclusion: Multiparametric flow cytometry by using comprehensive panel of monoclonal antibodies is a useful diagnostic test to evaluate body fluids or FNA as it can demonstrate small malignant populations that may be missed out by routine cytomorphology.

(73) Submission ID#807087

A New Primary Antibody Deficiency (PAD) Related Human Phenotype Ontology (HPO) Tree Facilitates the Formation of Homogeneous PAD-Patient Cohorts for Future Research

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Abstract/Case Report Text

The phenotypes of primary antibody deficient (PAD) patients range from milder (e.g. specific antibody deficiency) to severe (e.g. X-linked agammaglobulinemia) deficiency of the immune system. PAD patients form a clinically, immunologically as well as genetically heterogeneous group. Often, the genetic background has not been elucidated; it probably is not monogenetic in a large subgroup of patients. PAD patients suffer most frequently from recurrent bacterial infections of the respiratory or gastrointestinal tract due to immune deficiency, but may also have varying degrees of autoimmune and lymphoproliferative comorbidities due to immune dysregulation.

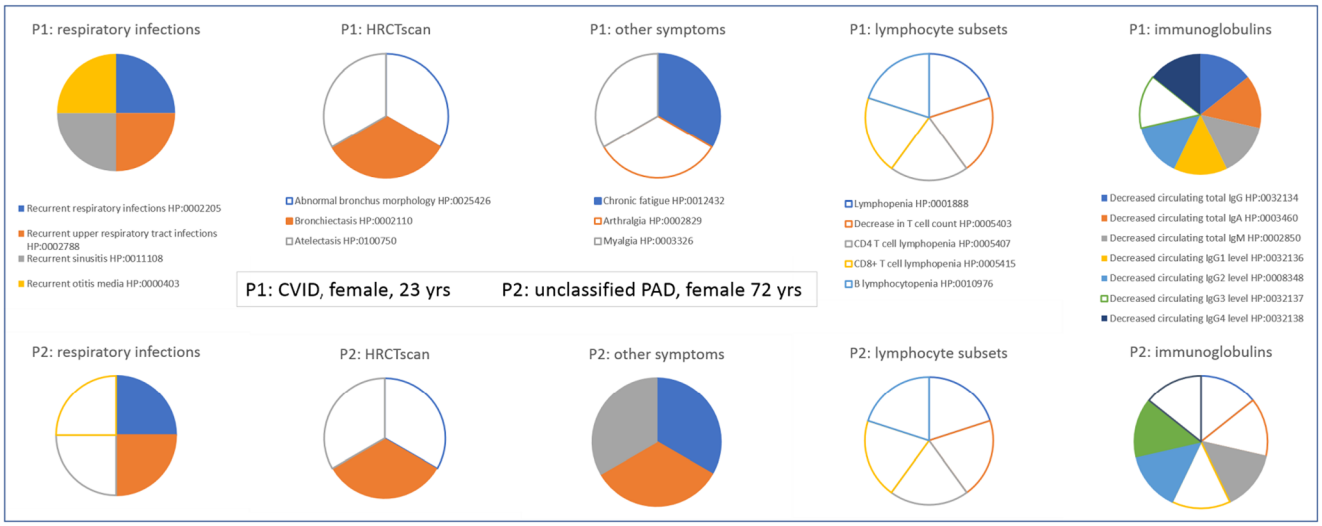
Unfortunately, a standardized description of PAD phenotypes is lacking rendering robust definitions of PAD-subtype diagnoses, including CVID, difficult. This impairs the formation of homogeneous cohorts that can form the starting point for future clinical and genetic research.

The PAD subgroup of the Human Phenotype Ontology (HPO) immune mediated disorders consortium supported by ERN RITA and ESID is addressing the gaps in standardized phenotypic description of PADs. Using the HPO dataset, literature mining, and ESID, IUIS and OMIM classifications, we aimed to re-evaluate and complete the PAD-related HPO terms to allow efficient data exchange and matching of phenotypically similar PAD patients.

As a principle, it was decided to avoid the ongoing variance in PAD-subtype definitions and to build the PAD-related HPO tree based as much as possible on unambiguously interpretable items. 'Hypogammaglobulinemia' was deleted as HPO term, and replaced by separate HPO terms such as 'decreased total IgG in blood', subdivided in 'transient' vs. 'chronic', and '(near) absent' vs. 'partially decreased' (the same for IgG1, IgG2, IgG3, IgG4, IgA and IgM). 'Decreased specific antibody level in blood' was specified further into 'decreased natural antibody level to blood group antigens in blood', subdivided in '(near) complete' vs. 'partial' absence (the same for protein, polysaccharide and protein-conjugated polysaccharide vaccination). Relevant HPO terms related to infection and to specific organ manifestations like bronchiectasis, autoimmunity and lymphoproliferation were re-evaluated and completed, and will be linked to PAD diseases in the HPO online system by the PAD subgroup experts.

Once finalized, existing PAD cohorts will be classified according to the new HPO PAD-related terms, and studied by clustering technologies (example of two patients shown in Figure 1; white = absent, color = present).

Acceptance and widespread use of this PAD-related HPO tree for standardized phenotyping will be essential to empower future multicenter clinical research and related genetic discoveries as well as support clinicians in diagnosing PAD through the linkage of HPO terms to PAD disease entities.



(74) Submission ID#807151

Ustekinumab in Leukocyte Adhesion Deficiency Type 1 (LAD1) Patients with Chronic Periodontitis

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Abstract/Case Report Text

Leukocyte adhesion deficiency type 1 (LAD1) is an autosomal recessive disorder characterized by the inability of granulocytes to emigrate from the bloodstream to sites of inflammation. LAD1 is caused by mutations in the ITGB2 gene (21q22.3), encoding the beta-2-integrin, CD18, which is essential for firm adhesion of leukocytes to the endothelium. In LAD1 survival is compromised, morbidity from inflammatory lesions is high, and treatment is poor. The moderate form of LAD1 is often managed with antibiotics for prophylaxis and during acute infections. After infancy severe gingivitis and chronic periodontitis are universal. Periodontal findings affect primary and permanent teeth, causing intense oral mucosal (gingival) inflammation and destruction of tooth supporting bone, which are hallmarks of the disease periodontitis.

Blocking the IL-23/IL 17 cytokines, which are up regulated in LAD1 gingiva, can reduce bacterial load and resolve inflammatory gingivitis. Ustekinumab binds to the shared p40 subunit of human IL-12 and IL-23, cytokines that modulate lymphocyte function, including T helper (TH) 1 cells and TH17 subsets, thereby blocking them.

Objective: Explore the effect of Ustekinumab on LAD1 inflammatory disease.

Method: Prospective study using Ustekinumab for oral inflammation. Patients receive five doses over 1 year, 45- or 90 mg depending of weight.

Results:

(Two patients have enrolled, P1 is >1 year post treatment, P2 is still on study)

Patient characteristics

· Patient 1: Age at diagnosis, 9yrs.(ITGB2 mutation c.2070delT (null)); CD18(%PMN control):32.6%; CD11a(%PMN);6.6%.

At the initiation of the protocol (17yrs old) WCC:7.41k/Ul; CRP: 6.10

· Patient 2: Age at diagnosis, 4yrs.(ITGB2 mutation c.850A>G,p.G284S c.809C>T,p.A270V)

CD18(%PMN control): 13%; CD11a(%PMN): 3.9%.

At the initiation of the protocol (17yrs old) WCC:7.41k/Ul; CRP: 6.10

Response:

Patient 1: Oral ulcers before treatment: episodes every two months.

During Ustekinumab therapy:

· Oral ulcers: 1 episode in a year
· Reduction in bleeding on probing: 57.5%
· Gingival Index Reduction: 95%

Patient 2: Oral ulcers before treatment : monthly.

During Ustekinumab therapy:

· Oral ulcers: none in first 4 months
· Reduction in bleeding on probing : 68.3%
· Gingival Index reduction: 37.5%

Safety:

No significant adverse events were documented during the therapy

P1 had a previous skin lesion that flared leading to IV antibiotics.

P2 had a previous sebaceous cyst drain spontaneously.

Discussion:

Two patients showed improvement in chronic periodontitis and a substantial decrease in oral ulcers while on ustekinumab. No clear safety signals were seen. Durability of these findings is still unknown. Ustekinumab in LAD1deficiency appears to be safe and potentially effective.

(75) Submission ID#807153

Expanded Phenotype Evaluation Of Patients With Primary Immunodeficiencies With Dysregulation

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Abstract/Case Report Text
Introduction: primary immunodeficiencies with dysregulation associate defects in the immune homeostasis leading to inappropriate immune response (lack or excess) that causes autoimmunity, allergy and/or inflammation. Impairment of different subsets of T and B compartments may be associated with these PIDs.
Aim: 1) Describe T and B memory compartment of 18 PID patients (pts) with dysregulation: 1 CD25 deficiency, 3 STAT1 GOF, 1 STAT5b deficiency, 4 CTLA4 variant, 2 PI3KCD variant and 6 CVID-like (with no molecular defect) and compare them with a group of healthy donors (HD). 2) Associate cTfh profile with B cell compartment impairment.
Results: 1) Pts showed a significant decrease of Naïve CD4+ T cells (CD45RA+CD27+) (52,2% vs 17,6%) ($p < 0.0001$) with expanded central memory T cells (CD45RA-CD27+) (32.8% vs 60.8%) ($p < 0.0001$); CD4+ T cells had higher levels of activation markers (CD4+HLA-DR+) (6,1%vs 27,4%) ($p < 0,0001$). Pts showed a significant increase of circulating follicular T cells (cTfh) (CD45RA-CXCR5+) compared with HD (mean 30,5% vs 11,6%) ($p < 0,0001$) with PD-1 overexpression ($p < 0.0001$). STAT1 GOF, CTLA4, PI3KCD and CVID-like pts showed a skew towards cTfh1 (CXCR3+). Regulatory T cells (CD4+CD25++ FOXP3+) were absent in CD25 and STAT5b deficiency and decreased in the other pts. Within CD8+ cells, although effector memory (CD45RA-CD27-) ($p < 0.002$), TEMRA (CD45RA+ CD27-) ($p < 0.009$) and HLA-DR+ CD8+ ($p < 0.002$) subsets showed a significant increase compared with HD, the behaviour was variable between different mutations. Regarding B cell compartment, pts with STAT1GOF, PI3KCD and CVID-like showed a severe impairment of switched-memory B cells (Sw-MBL) (CD27+IgD-IgM-); the STAT5b deficient patient had increased frequencies of this subset, while CTLA4 pts had a variable B defect. 2) lower Sw-MBL values were significantly associated with lower values of cTfh17 cells ($p < 0.007$) ($r=0.6975$). CD21low B cells were exclusively high in CVID-like pts, and transitional B cells were increase in PI3KCD and almost all CVID-like pts.
Discussion: In summary, patients with dysregulatory syndromes associate a defect of T and B homeostasis (survival, activation and differentiation). Specific mutations can differentially affect the quantity and/or the quality of cTfh. There is a strict association between the differentiations of Tfh with TH17 profile with the generation of Sw-MBL. These alterations may play a role in the pathophysiology of primary immunodeficiencies with B lymphocyte functional impairment. Immune monitoring of lymphocyte subsets of patient with dysregulation may approach to the diagnosis of specific monogenic mutations.

(76) Submission ID#807168

Identifying Primary Immune Deficiencies in Patients with Autoimmune Cytopenias and Biomarkers of Disease Activity

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Abstract/Case Report Text
Objective: The purpose of this study is to increase awareness and improve diagnosis of primary immune deficiency (PID) in the heterogenous group of patients with autoimmune cytopenia (AIC) by identifying clinical characteristics and laboratory biomarkers that distinguish those with underlying PID, disease activity and guide mechanism-based targeted therapy.
Methods: Patients with AIC (autoimmune hemolytic anemia (AIHA), immune thrombocytopenia (ITP), or autoimmune neutropenia (AIN)) were referred to our Immune Dysregulation team and prospectively enrolled during 2016-2019. Detailed immune phenotyping (IgG, IgA, IgM, lymphocyte subsets, vaccine titers, lymphocyte proliferation to mitogens/antigens), serum lipopolysaccharide (seLPS) and autoantibodies were measured and/or collected by chart review and genetic testing for PID was pursued.
Results: From 2016 to 2019, 93 patients were enrolled; two subjects were removed due to parental request or lack of AIC diagnosis. Of the 91 remaining patients, 43 (46%) were classified as "AIC-PID" based on genetic testing and/or immune phenotyping; 41 (45%) were classified as AIC-only, and 7 (9%) were asymptomatic family controls. The patients were predominantly children (ages 1-82 years, average age 17.3 years); 47% (44/93) were male. Among patients who have had genetic testing to date (n=66)(72%), pathogenic genetic mutations were confirmed in 23/66 (35%) of patients. Mutations include FAS/FASL (n=8, including 3 family members without AIC), CTLA4 (n=4), 22q11 (n=4), and one patient each with NFKB1, WAS, POLE-1, PI3K, CASP10, CARD11, and CGD; the remainder of AIC-PID patients were classified as combined immune deficiency or common variable immune deficiency based on immune phenotyping. Lymphocyte subsets (CD4+T, CD8+T, CD19+B, CD56+ NK) and immune globulins (IgG, IgA, IgM) tended to be lower in AIC-PID patients vs AIC-only ($p < 0.05$). Evans syndrome was more common in AIC-PID patients (13/43, 30%) compared to AIC-only (4/41, 10%). LPS was elevated in the serum of AIC patients compared to healthy controls (mean 719 vs 87 pg/mL, $p < 0.001$). Excluding partial DiGeorge syndrome patients (average LPS 222pg/mL), seLPS levels were significantly higher in genetically-defined untreated PID patients (average 1463 pg/mL) vs. other PID (average 444 pg/mL)($p=0.02$) or patients with AIC alone (average 667 pg/mL)($p=0.03$). Studies are ongoing on specific subsets that are linked to immune dysregulation (switched

memory B cells, T-regulatory cells, double negative T cells, T follicular helper cells) and the use of soluble IL-2 as a biomarker of disease activity.
Conclusions: A high fraction of AIC patient were identified with underlying PID in our study. Basic immune evaluation with immunoglobulin levels and lymphocyte subsets expedited diagnosis of PID. Genetic evaluation distinguished a group of patients with AIC-PID and highly elevated LPS level, reflecting high bacterial load, which may distinguish them from the rest the AIC cohort. The source of bacterial LPS can be multifactorial and is yet to be determined. Our studies continue focusing on biomarkers that can be applied to the heterogenous group of patients with AIC. This will allow early detection and timely initiation of targeted therapies.

(77) Submission ID#807260

APECED Rash as the First Manifestation of the Syndrome in a 10-Month Old Girl

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Abstract/Case Report Text

Introduction/Background: Autoimmune-Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy (APECED) is a monogenic autoimmune disease resulting from biallelic mutations in the AIRE gene. Although typically characterized by the classic triad of chronic mucocutaneous candidiasis, hypoparathyroidism and adrenal insufficiency, we recently reported that the clinical spectrum of the syndrome is far broader and that incorporation of an adjunct triad of APECED rash, intestinal dysfunction, and enamel hypoplasia in the classic triad could lead to earlier diagnosis (Ferre et al., JCI Insight, 2016). Among the adjunct triad manifestations, APECED rash occurs in 66% of American APECED patients by age 3, most often developing in the first year of life.
Objectives: To report and describe the clinical features of APECED rash as the first manifestation in a 10-month old patient with APECED.
Methods: Following enrollment in a NIAID IRB-approved protocol (11-I-0187) the patient was evaluated with history and physical examination, AIRE sequencing, measurement of interferon- autoantibodies, and skin biopsy with immunohistochemical analyses.
Results: A 10-month-old girl with a family history of genetically confirmed APECED in her 13-year old sister developed discrete circular, maculopapular erythematous lesions on her torso that spread to the face, arms, and legs while sparing the palms and soles. The rash was partially blanching, non-painful and non-pruritic and was preceded by low-grade fever (38□C) without other accompanying symptoms. She had not received medications or vaccinations prior to the rash onset. The lesions increased in size with associated central clearing and resolved 2.5 months after onset. The rash recurred with similar appearance 10 times over 14 months with each recurrence lasting between 10 days and 2.5 months. As with the first rash episode, recurrences were often preceded by fever (38-39□C) without accompanying symptoms or inciting factors. Neither topical nor oral antihistamines improved the rash.
AIRE sequencing identified the same compound heterozygous mutations (c.967_979del13 and c.769C>T) that the sister has. High titers of interferon-□ autoantibodies were measured in serum. Skin biopsy revealed superficial perivascular chronic inflammation and intraepidermal lymphocytes composed predominantly of mixed CD4 and CD8 T lymphocytes with few perivascular B lymphocytes. No eosinophils or vasculitis was observed. Myeloperoxidase immunostaining revealed extensive karyorrhexis. Laboratory studies revealed normal white count and ESR, negative anti-IgE receptor antibody, and positive anti-IgE antibody.
At 22 months, she developed oral candidiasis as second manifestation of APECED, thus reaching a diagnostic dyad when applying our proposed expanded diagnostic criteria. She has not developed hypoparathyroidism or adrenal insufficiency; thus, she has not yet reached a classic diagnostic dyad. Systematic screening for these endocrinopathies will be needed to avoid life-threatening complications of acute endocrine failure.
Conclusions: We report the clinical and histologic features of APECED rash manifesting as the first disease component of APECED in a 10-month old girl. APECED should be considered in the differential diagnosis of recurrent erythematous maculopapular urticaria-like eruptions characterized by mixed lymphocytic and neutrophilic infiltration unresponsive to antihistamines. Our case illustrates the clinical utility of incorporating the expanded diagnostic criteria of APECED rash, enamel hypoplasia and intestinal dysfunction into the classic diagnostic triad, which can lead to earlier APECED diagnosis.

(78) Submission ID#807294

Case of Persistent Neutropenia in a Patient with Hyper IgM Syndrome

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Abstract/Case Report Text

Patient is a 22 yo with h/o Hyper IgM Syndrome (hemizygous frameshift mutation in CD40L gene, c189delT) recently transitioned from the Children's Hospital of Philadelphia (CHOP) to the Hospital of the University of Pennsylvania (HUP) who presented with prolonged severe neutropenia despite G-CSF and seven hospitalizations for febrile neutropenia in the span of ten months. Prior to his neutropenia, patient was on monthly IVIG, with IgG trough 700-900 in the past year. He was evaluated for BMT in 2015 but declined.
Patient was first found to be neutropenic in Aug 2017 when he was admitted with Pseudomonas thigh abscess, HSV stomatitis and rhinovirus infection. He was treated with broad-spectrum antibiotics with improvement in neutropenia. The following month, he was hospitalized again with neutropenic fever, left axilla Pseudomonas abscess and rhinovirus infection. He underwent bone marrow biopsy revealing left shifted myeloids with decreased maturing forms and T cell predominant lymphoid aggregates, suggestive of autoimmune neutropenia vs. hyper IgM syndrome associated with neutropenia. Anti-neutrophils antibodies were negative. He was then admitted the following month (10/2017) with febrile neutropenia with CXR concerning for viral pneumonitis vs. atypical pneumonia. He was started on G-CSF therapy with significant initial response in ANC. However, this response was short-lived as he was again admitted in 12/2017 with febrile neutropenia and upper respiratory rhinovirus infection. He was continued on daily G-CSF.
Due to persistently normal ANC for approximately three weeks, he was weaned off G-CSF in 1/2018. In 3/2018 and 4/2018, he had two more hospitalizations for febrile neutropenia. G-CSF was restarted with dose uptitrated to 8mcg/kg during his hospitalization in April. He was found to be thrombocytopenic with splenomegaly on abdominal ultrasound. Anti-platelet antibodies

and repeat anti neutrophil antibodies were not detected. He was discharged with close follow up with Immunology and Hematology. Due to his age, he was transitioned to Penn Allergy/Immunology in 4/2018. There was close communication between CHOP Allergy/Immunology, CHOP Hematology and Penn Allergy/Immunology during this transition period.
Patient was admitted to HUP in 5/2018 with febrile neutropenia (despite higher dose of G-CSF), rhinovirus infection, Pseudomonas sinusitis and CT chest findings suggestive of possible fungal pneumonia. Due to persistent neutropenia refractory to G-CSF treatment, Hematology was consulted and repeat bone marrow biopsy showed hypercellular bone marrow with markedly left shifted granulocytic hyperplasia, compatible with G-CSF therapy. Flow cytometry showed no evidence of plasma cell neoplasm. Dose of IVIG was adjusted and increased based on his weight. Per hematology, he also received an additional high dose IVIG 1g/kg x 2 days for presumed immune mediated neutropenia with immediate increase in ANC. Despite ANC of 0 for 8 days, an additional 1 g/kg of IVIG improved his ANC to >1000 within 12 hours of his first dose. Thrombocytopenia also improved to normal range. Since then, patient has been on monthly 500-600mg/kg IVIG with no recurrence in neutropenia. This patient's prolonged persistent neutropenia with immediate response to high dose IVIG is suggestive of autoimmune neutropenia, which should be taken into consideration in Hyper IgM patients with persistent neutropenia.

(79) Submission ID#807329

The Association Between T-cell Immune Deficiency and the Risk of Serious Infection in Children with Thymic Hypoplasia Cared for at Duke University

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Abstract/Case Report Text
Background: Children with DiGeorge Anomaly (DGA) represent a heterogenous group, often classified as either partial DGA (pDGA) or complete DGA (cDGA) based upon the degree of thymic hypoplasia. This paucity of T-cell parameters and function has serious implications for infection risk, autoimmunity, and malignancy. However, there are limited studies stratifying children with DGA by these subgroups, especially in regard to immune function and subsequent infection risk.
Study design: Single-center, retrospective cohort analysis evaluating the relationship between pDGA and cDGA to infection risk with particular focus on infection-related hospitalization, pathogenic organism identification, and antimicrobial resistance profiles. The source population includes all pediatric patients < 18 years of age diagnosed with either pDGA or cDGA while receiving care at Duke University from January 1, 2014 to June 30, 2019. The final analysis sample included 145 patients.
Methods: To evaluate the differences in immune function between DGA subgroups, we will report the proportion of low (< 10th percentile for age) T cell immune biomarkers for both subgroups and compare populations using a chi-squared test. To compare per year incidence of infection-related hospitalization for DGA subgroups, a Poisson model with number of hospitalizations per patient as the outcome, an offset equal to the time at risk for hospitalization, and either pDGA or cDGA diagnosis as the exposure will be used. Models will be bivariate. We will report an incidence rate ratio (IRR) and 95% confidence interval (95% CI). To evaluate the impact of cellular and humoral immune function on infection-related hospitalization, we will use Poisson models where the outcome is the number of hospitalizations per patient, an offset equal to the time at risk for hospitalization, and low immune biomarker as the exposure. All models will be bivariate. We will report an IRR and 95% CI. Infection type and resistance profiles will be completely descriptive.
Results: As expected, children with cDGA had a significantly higher probability of a low (< 10th percentile for age) values for total T cells (CD3+), helper T cells (CD3+CD4+), cytotoxic T cells (CD3+CD8+), and naïve helper T-cells (CD4+CD45RA+CD62L+) as well as a significantly lower probability of low pan memory T-cells (CD3+CD45RO+) compared to children with pDGA. No differences were detected in the percentage of low natural killer (NK) cells (CD15+CD56+) or B cells (CD19+) between subgroups. cDGA patients had a significantly higher incidence of hospitalization per year (1.52 (0.61, 3.80)) compared to pDGA patients (0.20 (0.12, 0.32)). The IRR is 7.62 (2.71, 21.3). Across both subgroups, the incidence of hospitalization was higher in DGA patients who had low helper and naïve T-cells. There is ongoing analysis into hospital-related infection and resistance profiles. Notable frequencies include bacteremia (> 5%), invasive viral disease (> 1%), and opportunistic infections (> 1%).
Conclusions: Children who had cDGA were 662% more likely to have an infection requiring hospitalization than children who had pDGA, emphasizing the need for thymus transplant for cDGA. Further analysis of infection type and patient outcomes is critical to enhancing management of this unique patient population.

(81) Submission ID#807404

Zika Virus Antibody-Positivity Among Symptomatic/Asymptomatic Pregnant Women In Aseer Region Displays Pre-Exposure To Other Cross-Reactive Flavi Viruses

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Abstract/Case Report Text
Antibody cross-reactivity among flavivirus has been documented. In recent times Zika virus has been emerging in pockets of the mosquito-infested regions, While Southwestern Saudi Arabia is known for Arthropod-borne viral diseases and we do not know the incidence or even presence of Zika virus in this region. It is restricted to predict the IgM and IgG antibody detection ranges owing to limited data and colossal cross-reactivity among the Zika and other flaviviruses. We tested sera from 217 pregnant women irrespective of their clinical presentation for Zika and Dengue IgM, IgG respectively. The Zika positive samples were further confirmed by Plaque reduction neutralization tests (PRNT). From our results, 3.6% (8) cases were positive for ZIKA IgM against 1.8 % (4) positivity to IgG. When these samples were assessed for Dengue IgM and IgG, we observed 1.3% (3) seropositivity for IgM and IgG respectively. There was no single sample positive for both IgM and IgG of Zika or Dengue. However, we observed one sample positive for both Zika and Dengue IgM. Upon mapping the overlapping serotiters, there was no significant correlation observed between the Dengue IgM

and IgG. Whereas Zika IgG positive sample showed high serotiter for Dengue IgG indicating the contribution of cross-reactivity for observed Zika positivity. Screening for the incidence of Zika, therefore, becomes particularly hard in a population that has the presence of pre-exposure of Dengue and this cross-reactivity makes it hard to determine the Zika incubation and antibody prevalence confounded with other flaviviruses.

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One Year Experience Using S. TYPHIM Vi Vaccine To Assess PID In Adults

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Abstract/Case Report Text

INTRODUCTION Humoral PID diagnostic protocol includes the analysis of the immune response to different protein and polysaccharide antigens (Ags)(1). Although the analysis of the immune response against the polysaccharides vaccine from Pneumococca has been the standard method, the use of S. Typhim Vi vaccine has appeared as a good alternative (2). In this report we show the results obtained with the use of S.Typhim Vi in 27 adults patients attending the PID outpatient clinic.

MATERIAL AND METHODS Patients with humoral-suspected PIDs were challenged with Typhoid polysaccharide vaccine (Typhim Vi®; Sanofi-Pasteur). Serum was obtained on basal and after 4 weeks of vaccination. Specific IgG levels against S.typhim were measured using "VaccZyme TM Human Anti-Salmonella typhi Vi IgG Enzyme Immunoassay Kit" (Binding-Site).

RESULTS A total of 90 Adult patients attended the PID clinics during Nov 2018-Nov 2019. From those, 27 patients were fully evaluated using a Humoral-Suspected PID algorithm that includes the S.Typhi vaccination. In total 13 male and 14 female patients completed the protocol and were analyzed. Twenty patients were considered as responders (ratio pre/post >3x) whereas 7 patients were non-responders.

DISCUSSION The main advantage of assessing polysaccharide immune response using S.tyhpim is the usual lack of specific IgG at the moment of the initial evaluation. In this serie, just one patient has a high basal level (#2-vaccinated in the past). Thirteen patients had basal levels below the detection limit of the test (7,4U/mL) and 13 patients between 7,4 and 23,35U/mL, that has been described as a cut otf level for non-immunised individuals (personal experience,3,4).

Regarding the polysaccharide immune response as a tool to distinguish PID vs non-PID patients, the results showed a good correlation between those non-responders with more clinical relevant PID diagnostics. Seven non-responders patients were subsequently diagnosed with a primary (* on Table I) and/or secondary ID (** on Table I). Despite this, there were 16 patients that we could have classified as Strong Responders (ratio >3x, absolute specific IgG post-vaccination level >100U/mL) and 4 patients considered as Weak Responders (ratio Post/Pre >3x, absolute specific IgG post-vaccination level < 100U/mL).

Strong responders were considered non-PID after including other clinical investigations and laboratory tests (Cell subpopulation study, PCP response) whereas weak responders group consisted in some "minor" forms of PID, like isolated IgM or Ig subclasses deficits. More patients are needed to confirm this functional classification of PID patients regarding their S.Typhi immune response.

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Table I: Polysaccharide Immune Response of 27 Patients attending the PID Adult Clinic Nov2018 -Nov2019

	Age	Gender	Sal-Pre	Sal-Post
1	35	Male	11.21	210.82
2	72	Female	51.18	> 600.00
3	68	Female	13.50	> 600.00
4	60	Male	21.35	> 600.00
5	49	Female	10.08	598.20
6	56	Male	11.77	> 600.00
7**	60	Female	< 7.40	8.50
8	57	Female	11.49	> 600.00
9**	65	Female	< 7.40	10.00
10	28	Male	23.26	326.30
11	53	Female	< 7.40	84.70
12	48	Male	< 7.40	278.60
13	26	Male	7.82	41.50
14	24	Male	11.77	142.50
15	21	Male	10.93	> 600.00
16*	59	Female	< 7.40	8.34
17	64	Male	< 7.40	32.38
18	58	Female	< 7.40	267.20
19*	70	Female	< 7.40	< 7.40
20*	38	Female	< 7.40	10.20
21*	39	Female	< 7.40	< 7.40
22	70	Male	7.54	38.79
23	49	Male	15.43	295.20
24	80	Female	< 7.40	120.35
25	53	Female	8.54	141.60
26	40	Male	< 7.40	363.90
27*	33	Male	<7.40	< 7.40

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CVID And Dysregulation Syndrome In Three Members Of A Same Family With A New Variant Of CTLA4 Gene

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Abstract/Case Report Text

Introduction: CTLA4 is a negative immune regulator that inhibits Tcell activation by various suppressive functions. Haploinsufficiency in CTLA4 presents with a diverse clinical phenotype and laboratory results. Objective: Report a family with diverse clinical presentation due to CTLA gene variant.Results: Three members of a family presented a CTLA4 gene variant. The index case (IC) is a girl 15 years old (yo), with a background of severe varicella and mononucleosis syndrome during her childhood. Since 11yo suffered from recurrent oral thrush and Evans Syndrome. She was treated with IVIG, steroids and rituximab, without improvement. Luckily, cytopenias were controlled by Eltrombopag and Sirolimus. Laboratory showed: low IgM, IgG and antibody responses could not be evaluated because of IVIG; T/NK lymphopenia, with an expansion of central memory T cells. Her sister (18yo) has recurrent respiratory infections (RRI), since she was 1yo and has been suffering from chronic diarrhea. A bowel biopsy (BB) demonstrated follicular lymphoid hyperplasia (FLH) and focal active colitis. IBD showed partial response to steroids (discontinued) and azathioprine. Vedolizumab was added. She was diagnosed with CVID due to hypogammaglobulinemia and impaired antibody responses. Moreover, she showed T/NK lymphopenia, low counts of Post-switch B cells and an increase of TCR αβ double negative T cells. Actually she is under SCIG. Their father (47yo), has been suffering from RRI since his childhood and oral/genital thrush. He developed a severe goiter at 18yo. For the past 10 years, he has been switching between diarrhea and constipation. FLH was found in BB, therefore oral Budesonide was started. He only showed mild T-Lymphopenia. All of them present low levels of regulatory T cells (Treg) and high levels of circulating T follicular helper cells (cTfh) with a variable cTfh1/cTfh17 profile. NGS showed an heterozygous CTLA4 gene variant (p.Leu141Pro). This is not reported in ExAC or GenomAD. Futhermore, the three patients had low expression of CTLA4 in Tregs. We believe that this finding could explain the different phenotypes. Both siblings received a Hematopoietic stem cell transplantation (HSCT): IC with match unrelated donor, and her older sister with match related donor. Both received a reduced-intensity conditioning (RIC) regimen based on melphalan and fludarabine associated with antithymocyte globulin (ATG). Good neutrophils engraftment. Currently, they are in day + 19 IC and in day + 27 awaiting immune reconstitution. Discussion: The 3 patients presented symptoms in second childhood and adolescence. Their clinical manifestations were different. Both daughters with CVID profile due to hematological and intestinal involvement and endocrinological involvement in their father, All with low CTLA4 expression in Treg. This demonstrates the complexity of these patients that lead us to face different therapeutic paths from the modulation of the treatment to a BMT. HSCT for severe presentation can be a therapeutic option. However, there are few published reports in the literature. The great risk in patients who undergo to HSCT is inflammation because of the alloreactivity development

(84) Submission ID#807577

APDS2 and SHORT Syndrome in a Teenager with PIK3R1 Pathogenic Variant

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Abstract/Case Report Text

Activated PI3Kδ syndrome (APDS) is a primary immunodeficiency characterized by recurrent respiratory infections, as well as increased risk of chronic viremia with herpes family viruses, benign lymphadenopathy and B cell lymphoma. It is caused by heterogeneous germline gain-of-function mutations which ultimately lead to the hyperactivation of the phosphoinositide-3-kinase δ (PIK3δ). PIK3δ exists as a heterodimer composed of a catalytic and a regulatory subunit. It interacts with B cell receptors, T cell receptors, costimulatory and cytokine receptors, and is a key player in a signaling pathway involved in cell growth, proliferation and survival. APDS1 is caused by mutations in the PIK3CD gene, affecting its protein product p110 δ (catalytic subunit). APDS2 is caused by mutations in the PIK3R1 affecting p85a (regulatory subunit).

SHORT syndrome is a rare multisystem disorder characterized by short stature, hypertextensible joints, ocular depression, Reiger anomaly and tooth eruption delay. The primary causes of SHORT syndrome are heterozygous loss-of-function mutations in the PIK3R1 gene. The combination of APDS2 and SHORT syndrome is very rare, with only few cases described in the literature. In this report we present a teenager with a pathogenic variant in the PIK3R1 gene, and phenotypic characteristics of both APDS2 and SHORT syndrome.

Our patient is a 17-year-old female with a history of growth delay and short stature, delay tooth eruption, recurrent sinopulmonary infections and hypogammaglobulinemia. Evaluation performed at a prior institution for recurrent infections revealed low IgG levels. She did not initiate therapy at that time and was lost to follow up for several years. At the time of our initial evaluation she reported continued recurrent episodes of upper respiratory infections and sinus infections requiring antibiotic treatment that often did not clear the infections. Her physical exam was relevant for short stature (1%ile, z=-2.33), low weight for age (< 1%ile, z=-2.69) and hyperextensibility. Her facial features were significant for prominent forehead and triangular face.

Given concern for immune deficiency, a complete immune evaluation was obtained. Her workup revealed low IgG levels, with IgM and IgA within normal limits. She did not have protective titers to S. pneumoniae, H. Influenza or Diphtheria and Tetanus. After administration of vaccine boosters, she was able to generate a response to all vaccines except for Tetanus. She had remarkably low absolute B cells (34 cells/uL) and percentage (1 %), and low CD4:CD8 ratio (0.84). She was started on Amoxicillin prophylaxis and monthly IVIG replacement therapy. Invitae immunodeficiency panel genetic testing was sent and revealed a pathogenic loss of function variant in an intronic splice site in the gene PIK3R1 (c.1425+1G>C). After initiating treatment with IVIG, her sinus infections significantly improved and she has not had any further episodes. IgG levels have remained within normal limits with monthly IVIG therapy.

This pathogenic variant had been previously associated with APDS2; however, it had not been associated with SHORT syndrome. The mechanisms that link both conditions is yet to be identified. This case report emphasizes the importance of screening for comorbidities associated with SHORT syndrome in APDS2 patients, and vice versa.

(85) Submission ID#807746

Diagnostic Findings In Patients Suspected Of Primary Immunodeficiency And Custom Analysis Of NCF1 To Improve Diagnostic Yield In Patients With Chronic Granulomatous Disease

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Abstract/Case Report Text
Finding the genetic diagnosis for patients with suspicion of primary immunodeficiency (PID) is becoming increasingly important in the management of primary immunodeficiency and estimating the risk for family members. We constantly increase the diagnostic yield for PIDs by improving the sequencing technology, updating the panels with new genes discovered related to PID, and finding diagnoses from difficult to sequence regions and regions with high homology. Here we report our experiences with nearly 1700 patients suspected with PID. Moreover, we provide a case example, how we increase the diagnostic yield by developing unique techniques for specific genes which cannot be reliably analyzed by NGS alone.
Diagnostic yield including all immunology related panels was 14.9% (253/1698). The majority of the tested individuals were males (1343/1698, 79.1%) and the most common age of testing was between 10 to 20 years (468/1698, 27.6%). The highest diagnostic yield 20.6% (75/364) is in children from ages 0 to 5 years, whereas in patients over 60 years of age the diagnosis was found for only 6.5% (4/62) of the patients. The diagnostic yield was highly variable between the different panels; diagnostic yield for Primary Immunodeficiency Panel was 13.3% (138/1036), whereas for Severe Combined Immunodeficiency Panel it was 46.7% (7/15), and for Bone Marrow Failure Syndrome Panel 21.4 % (36/168). Out of the 296 reported diagnostic sequence variants, 234 (79%) were unique, and only 7 diagnostic variants were reported over 3 times. Copy number variants (CNVs), including deletions and duplications, were reported for 19 patients from 17 different genes.
The diagnostic yield for Chronic Granulomatous Disease (CGD) Panel is high (10/21, 48%) although analysis of the NCF1 gene included in this panel is complicated by two highly homologous pseudogenes NCF1B and NCF1C. Deletion calling from the NGS data cannot well identify or distinguish potential deletion in the pseudogene or the actual NCF1 gene. In two patient cases, our CNV detection algorithm indicated a homozygous deletion in the index patient samples potentially covering the whole NCF1 gene. Additional bioinformatic analysis targeting specifically two coding positions that differ between the NCF1 gene and the two pseudogenes showed that all reads in those positions originated from the pseudogenes. Homozygous deletion in the NCF1 gene was further confirmed by Sanger sequencing two regions in NCF1 with primers that specifically bind to either NCF1 or the pseudogenes. While clean NCF1 sequences from both regions were obtained for a control sample, no NCF1-specific amplification product was obtained for the index patient samples. Pseudogenes were amplified and sequenced successfully in both index patient samples and positive control samples. Loss-of-function of NCF1 is a well-established mechanism leading to CGD and by overcoming the difficulties regarding NCF1 deletion detection by NGS, we can improve diagnostic rate in individuals affected with CGD.

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Defining Novel Populations of Circulating NK Cells in Human GATA2 Deficiency

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Abstract/Case Report Text
Human innate lymphoid cells, including natural killer cells, play a critical role in the control of viral infection and malignancy. GATA2 deficiency can lead to a broad spectrum of clinical and hematological phenotypes; in some cases, NK cell deficiency is the primary manifestation, resulting in a greatly increased susceptibility to viral infections and malignancy. GATA2-deficient patients, particularly those who suffer from severe viral infections, have reduced frequencies of peripheral blood NK cells and loss of function in the existing NK cells. Specific loss of the less mature (CD56^bright) NK cell subset is a hallmark of the immune phenotype in GATA2 deficiency, suggesting that generation or survival of NK cell precursors is impaired. Given the remarkable spectrum of clinical phenotypes in GATA2-deficient patients and the poorly understood biology underlying their NK cell defect, we sought to characterize circulating NK cells on a single-cell level.
We performed single-cell (sc)RNASeq of lineage-depleted innate lymphocytes from a patient with GATA2 deficiency. As expected from flow cytometric phenotyping of peripheral blood cells from this and other patients, scRNASeq revealed decreased representation of canonical CD56^bright cells. Within the CD56^dim population, we identified two NK cell populations that were seemingly unique to the GATA2 deficient patient relative to a healthy donor. Pathway analysis defined the first of these populations (Population 1) by the expression of genes associated with cellular response to stress, extracellular stimulus and inflammation, as well as programmed cell death and regulation of proliferation and apoptosis. The second population (Population 2) was defined by genes associated with NK cell chemotaxis, cytokine responses and interferon signaling.
To extend our findings, we performed scRNAseq of 6 additional healthy donors and analyzed an additional GATA2-deficient individual who was clinically asymptomatic (Yang et al. 2019). Of note, we detected Population 2 in seemingly healthy CMV-negative individuals, suggesting it was not uniquely a result of GATA2 deficiency but associated with an inflammatory response and not related to adaptive NK cells generated in response to CMV

infection. Population 1, on the other hand, only appeared in our symptomatic GATA2-deficient patient. We additionally performed bulk gene expression analyses from an unrelated GATA2-deficient patient that confirmed the altered expression of genes associated with both novel cell populations. Current efforts are focused on better defining the functional response of NK cells in these patients and confirming the identification of our novel populations by mass cytometry (CyTOF). Together, our data define the heterogeneity and complexity of NK cells in GATA2 deficient and healthy individuals.

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Spontaneous Gastrointestinal Perforations In LOF STAT3 And The Role Of Impaired IL-6 Signaling

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Abstract/Case Report Text
Introduction: Dominant negative mutations in STAT3 (LOF STAT3) cause a hyper IgE syndrome characterized by recurrent skin and lung infections, eczema, mucocutaneous candidiasis, and connective tissue, vascular and skeletal abnormalities. Intestinal perforations, spontaneous and with diverticulitis, have been reported in LOF STAT3. Recently, spontaneous intestinal perforations have been reported with tocilizumab, a monoclonal antibody of the interleukin 6 (IL-6) receptor, suggesting that IL-6 signaling plays a role in intestinal wall integrity. As IL-6 signals through STAT3, we sought to investigate the potential association between LOF STAT3 and intestinal perforations, as well as the incidence and outcome in our patient cohort.
Methods: We performed a retrospective chart review of patients with LOF STAT3 (n=158) followed at our institution, looking for those with non-malignancy associated spontaneous gastrointestinal perforations. The demographic information, STAT3 mutation, comorbidities at the time of perforation, clinical presentation, management, and clinical outcomes were compiled
Results: Ten LOF STAT3 patients were identified as having documented intestinal perforations, an approximate rate of 6%. One perforation was the initial presentation of diffuse large B cell lymphoma (DLBCL) of the duodenum and liver, and was excluded from the rest of the analysis. The other nine perforations occurred between 4 to 60 years old (mean:26), and 55% were female. STAT3 mutations were localized to the DNA binding domain (n=4) and the SH2 domain (n=5). Two of the perforations occurred while inpatient for lung infection. Another occurred while recovering from pneumonia at home. Two perforations were associated with the initial diagnosis of diverticulitis (at age 26 and 60). One perforation occurred in the terminal ileum, one in the cecum, one in the transverse colon, and six in the sigmoid. Five patients underwent primary closure of the bowel. Four patients required a temporary ostomy, with subsequent successful ostomy reversal. Only one patient has since died of pulmonary hemorrhage, the other patients are alive with a mean of 6 years post perforation follow-up, with no recurrence of perforation. One patient with prior sigmoid resection required ileal resection 6 post perforation due to as massive intestinal bleed.
Conclusion: Spontaneous gastrointestinal perforations occurred in our LOF STAT3 cohort at a rate of approximately 6%. One case was associated with malignant infiltration of the gastrointestinal tract and two cases were associated with diverticulitis both known risk factors for perforation. Although the pathogenesis of the perforations in LOF STAT3 remains unclear, the connective tissue phenotype likely contributes as well as the association with diminished IL-6 signaling, as has been demonstrated with the perforations and tocilizumab.

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Rituximab-Azathioprine Or Rituximab-Mycophenolate for Treatment of Granulomatous and Lymphocytic Interstitial Lung Disease in Patients with Common Variable Immunodeficiency

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Abstract/Case Report Text
Background Granulomatous and lymphocytic interstitial lung disease (GLILD) is a life-threatening complication that occurs in patients with common variable immunodeficiency (CVID) and monogenic CVID-like disorders, but the optimal treatment is unknown.
Objective To determine if the use rituximab and azathioprine (RTX-AZA) or rituximab and mycophenolate mofetil (RTX-MMF) would improve the radiographic abnormalities as determined by high-resolution computed tomography (HRCT) of the chest and/or pulmonary function tests (PFTs) in patients with CVID and GLILD.
Methods This is a retrospective study of patients seen from July 2006 to December 2018 with CVID and GLILD who

completed immunosuppressive therapy (RTX (375mg/M2) for 4 weeks, repeated at 6-month intervals for 3 or 4 total courses, and AZA (0.5-2.0 mg/kg/day) or MMF (250mg-1000mg-BID) for 18 months). Complete PFTs and HRCT scans were performed prior to therapy, at the conclusion of therapy, and periodically thereafter. HRCT scans were blinded, randomized, and scored independently (in pairs) by two radiologists. All patients underwent whole exome sequencing (WES). Number (percentage) and median (interquartile range) were reported for categorical and continuous variables, respectively. Differences between pre- and post-treatment and between relapse and post-relapse HRCT scores and PFT parameters were analyzed with Wilcoxon signed ranks test. Kaplan-Meier survival curves were also done. Unadjusted one-sided p-values < 0.05 were considered statistically significant.

Results The GLILD cohort (N=39) had a 2:1 female predominance, and age at GLILD diagnosis was 36 (25-43) years (Table 1). Autoimmunity was present in the majority of patients, with thrombocytopenia (28 (72%)) the most common manifestation. Enteropathy (3 (8%)), inflammatory bowel disease (1 (3%)), and nodular regenerative hyperplasia of the liver (6 (15%)) were also present. Splenomegaly (31 (79%)) was present in the majority, but polyarthritis (0 (0%)) was notably absent. Twenty (51%) patients had been previously treated with systemic steroids.

HRCT scores substantially improved between pre- and post-treatment for RTX-MMF (p=0.0005) and RTX-AZA (p < 0.0001, Figure 1). FEV1 (p=0.019), FVC (p=0.0011), and TLC (p=0.0071) also improved, but DLCO (p=0.060) was unchanged (Figures 2 and 3). Excluding two (5%) patients who died 2.3 and 2 years after therapy of respiratory failure (1 (3%)) and septicemia (1 (3%)) respectively, 9/37 (24%) patients relapsed 3.2 (2.8-3.5) years following therapy with an estimated 60% relapse rate after 5 years (Figure 4). As of December 2018, 6 of 9 patients that relapsed showed improvement in HRCT scores (p=0.031), and the remaining 3 patients are still undergoing retreatment (Figure 5). Four (10%) pneumonias occurred during immunosuppressive therapy, all with severe restrictive lung disease . Eight (21%) patients had a damaging mutation in a gene known to predispose (TNFRSF13B, n=3 (8%)) or cause a CVID-like primary immunodeficiency (CTLA4: 2 (5%); KMTD2: 2 (5%); BIRC4: 1 (3%)). Immunosuppressive treatment improved the HRCT scores regardless of the absence (p < 0.0001) or presence of a damaging mutation (p=0.0039) (Figure 6).

Conclusion Combination chemotherapy appeared to be effective in improving the radiographic abnormalities and pulmonary function of patients with CVID and GLILD. A majority of patients had sustained remissions, regardless of the presence or absence of a monogenic disorder.

Characteristics at pre-treatment	CVID and GLILD (N=39)
Gender: n (%)	26 (67%)
Female	13 (33%)
Male	
Age at GLILD diagnosis, years: median (IQR)	36 (25-43)
Estimated duration of GLILD, years: median (IQR)	2 (1-6)
Age at CVID diagnosis, years: median (IQR)	29 (23-39)
Estimated duration of CVID, years: median (IQR)	8 (3-14)
Mode of Diagnosis: n (%)	35 (90%)
VATS	3 (8%)
Tbx	1 (3%)
MS	
Normal PFTs: n (%)	16 (41%)
Prior Steroids: n (%)	20 (51%)
H/O Cytopenias: n (%)	28 (72%)
Splenomegaly: n (%)	31 (79%)
Splenectomy: n (%)	5 (13%)
Liver dz/NRH: n (%)	6 (15%)
Enteropathy: n (%)	3 (8%)
IBD: n (%)	1 (3%)
Polyarthrits: n (%)	0 (0%)

IQR=interquartile range, CVID=common variable immunodeficiency, GLILD=granulomatous and lymphocytic interstitial lung disease, VATS=video-assisted thoracoscopic surgery, Tbx=transbronchial biopsy, MS=mediastinoscopy excluding B cell malignancy, PFT=Pulmonary Function Test, H/O=history of, dz=disease, NRH=nodular regenerative hyperplasia, IBD=inflammatory bowel disease

Table 1. Baseline patient characteristics

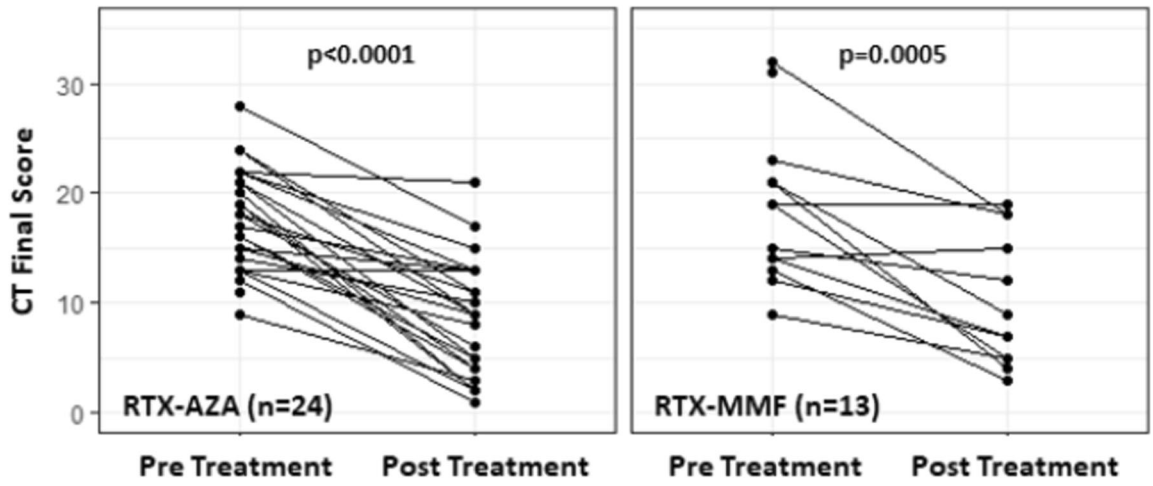

Figure 1 HRCT scores of the chest before and after therapy with RTX/AZA (p<0.0001) or RTX/MMF (p=0.0005).

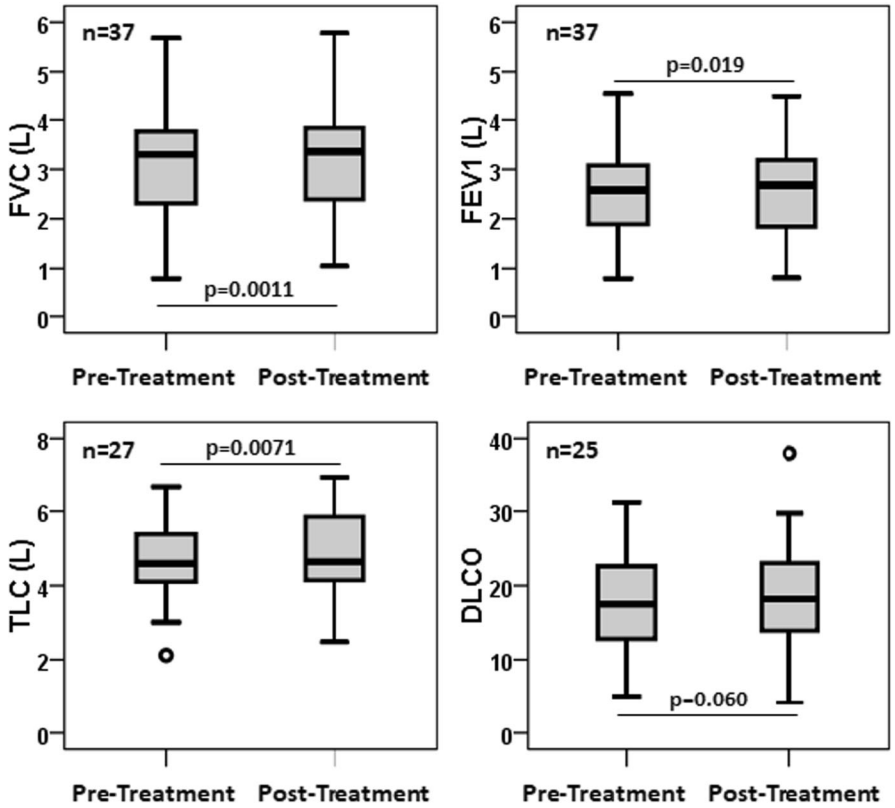

Figure 2 FEV1, FVC, TLC and DLCO before and after therapy with either RTX/AZA or RTX/MMF (FEV1 p=0.0011; FVC p=0.0019; TLC p=0.0071; DLCO p=0.060).

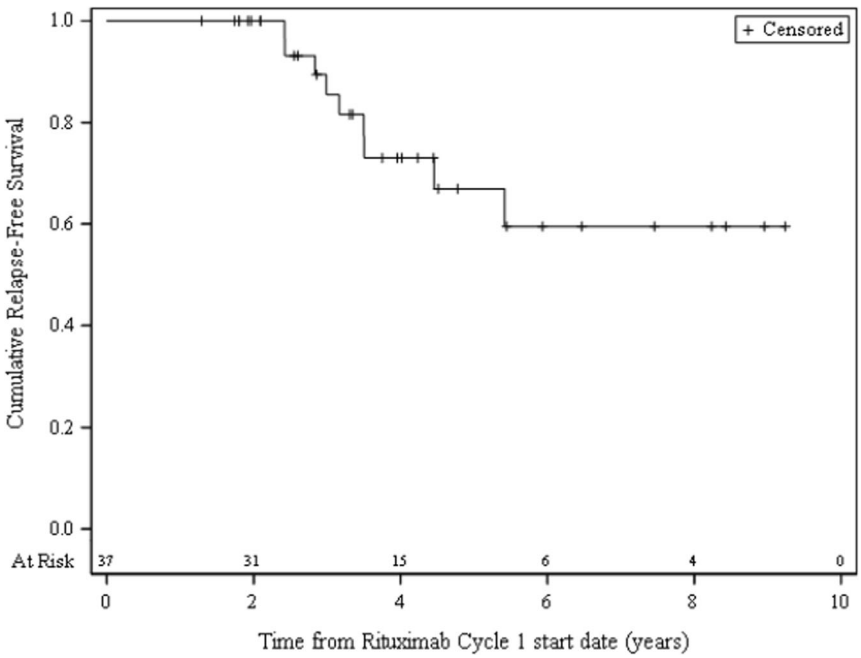

Figure 3. Kaplan-Meier disease-free survival curve after either RTX/AZA or RTX/MMF.

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A Novel Mutation in PI3KD Gene in a Patient with Chronic Systemic Inflammation And Recurrent Abscesses

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Abstract/Case Report Text

Introduction/Background: Gain of function mutations in PIK3CD gene have been associated with Activated PI3K Delta Syndrome. Since its first description the number of cases has increased progressively [1]. Although described as a predominant antibody deficiency[1], various complex phenotypes have been associated with mutations in this gene[2].

Case Description.

Female patient with no remarkable history until 2 years old, when she suffers from a persistent fever associated with purulent abscesses in venipuncture areas and hyperleukocytosis with neutrophilia, so she was treated for about 8 months in 3 hospitals in the city of Barranquilla before she was referred to our institution. The patient's clinical picture consisted of persistent fever unresponsive to broad-spectrum antibiotic treatments, skin abscesses, left subphrenic abscess and toes osteomyelitis. The microbiological studies documented a bacteremia by Acinetobacter baumannii and isolation in bone marrow of Candida parapsilosis. During her care stay in Barranquilla, she was approached as a chronic granulomatous disease versus Job's syndrome, she received two doses of immunoglobulin with partial control of symptoms. Due to the recurrence of fever, abscesses and hyperleukocytosis, they decided to refer to our institution for further studies. Upon admission to our institution, the patient presented nutritional compromise, with spontaneous resolution of fever but persistence of high acute phase reactants, with significant improvement of leukocytosis. All the cutaneous lesions she presented were debrided at the site of remission. Immunoglobulin levels, lymphocyte populations and dyhidrorhodamine test were normal. She remained with no weight gain, constipation, abdominal distension and hepatic involvement with elevated liver enzymes and prolonged coagulation times. New bone compromise was documented. Inflammatory bowel disease, neoplastic or chronic infectious disease involvement was ruled out. During the stay in our institution no microbiological isolation was documented. Skin, colon and bone tissue biopsies were performed and extra-institutionally performed liver biopsy were examined, showing as a single common finding leukocytoclastic vasculitis in all tissues. Given the heterogeneous nature of the condition, the diagnostic possibility of an immune dysregulation disorder was considered and a therapeutic trial with NSAIDs and prednisolone at 1mg/kg/day was started, as well as genetic studies by exome sequencing. The exome results documented a novel mutation in the PIK3CD gene [c.1895T> A (p.Phe632Tyr)] as probably pathogenic. The patient has presented a clinical improvement and a significant decrease in inflammation markers. At the moment, we are waiting for the performance of functional tests to define the definitive therapy for this patient.

Conclusions. This case description highlights the diagnostic difficulties that face in developing countries, where the non-availability of functional testing has implications on the diagnosis opportunity and establishment of optimal therapeutic for patients with complex diseases such as primary immune regulatory disorders.

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Cyclic Autoinflammatory Syndrome as Observed by Providers and Reported by Parents

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Abstract/Case Report Text

Background: Autoinflammatory syndromes, a wide family of diseases, defined as attacks of inflammation that are unprovoked (or triggered by a minor event) and are primarily related to dysregulation of the innate immune system. Periodic/recurrent fever syndromes were the former name of these diseases. However, only in two conditions: cyclic neutropenia (CN) and periodic fever, aphthous stomatitis, pharyngitis, and adenitis (PFAPA) are febrile episodes truly periodic. For PFAPA, although diagnostic criteria differ and there is no consensus research definition, patients are usually not difficult to recognize based on clinical course and presentation1. High index of suspicion and understanding the parental experiences and descriptions of febrile episodes is imperative in facilitating early recognition and timely diagnosis.

Aims: To standardize and summarized the clinical presentation of PFAPA, based on parental descriptions and providers observation of febrile episodes.

Methods: Utilizing a query for the ICD-10 diagnosis code M04.8(+) we identified a cohort of children diagnosed and managed for periodic fever, excluding those with monogenetic mutation (e.g. Blau, Majeed) and those with chronic illness. We reviewed the charts for documented parental report and provider observation of febrile episodes. Standardized signs and symptoms were recorded for each patient [Table 1].

Results: A cohort of 75 children, 35 boys (47%) and 40 girls (53%) with documented, cyclic episodes of fever >101, was identified. The average age at diagnosis was 3.77 +/- 2.34 years. Classic symptoms were reported or observed in 85% of patients (64). More than half (68%, 51 patients) had documentations of other symptoms, usually reported by parents to occur sporadically during some fever episodes. Decreased oral intake and general “ill appearance” was reported by parents in 91% of patients. When reporting the time intervals, parents usually reported similar length for each episode, typically between 3-5 days, and regular interludes, typically between 3-4 weeks. The findings are summarized in table 2.

Discussion: The findings presented here are in concordance with previously published data describing PFAPA as a syndrome affecting young, generally healthy children with identical episodes of fever lasting for a few days, recur with regularity. Our data support the approach that parental observation is fundamental in identifying the unique pattern of illnesses. Engaging the parents with directed interview is crucial to establish this clinical diagnosis in a timely fashion, prevent misdiagnoses of future febrile episodes as presumptive infections, and avert unnecessary antibiotics courses. This cohort adds the observation that up to 15% of patients who display this identifiable pattern of illnesses, do not present with aphthous stomatitis, pharyngitis, or adenitis. These classic symptoms although common, are not the rule. The cyclic chronicity of febrile episodes associated with general ill appearance (but not lethargy), and decreased PO, in an otherwise healthy child is the clinical gold-standard of this condition.

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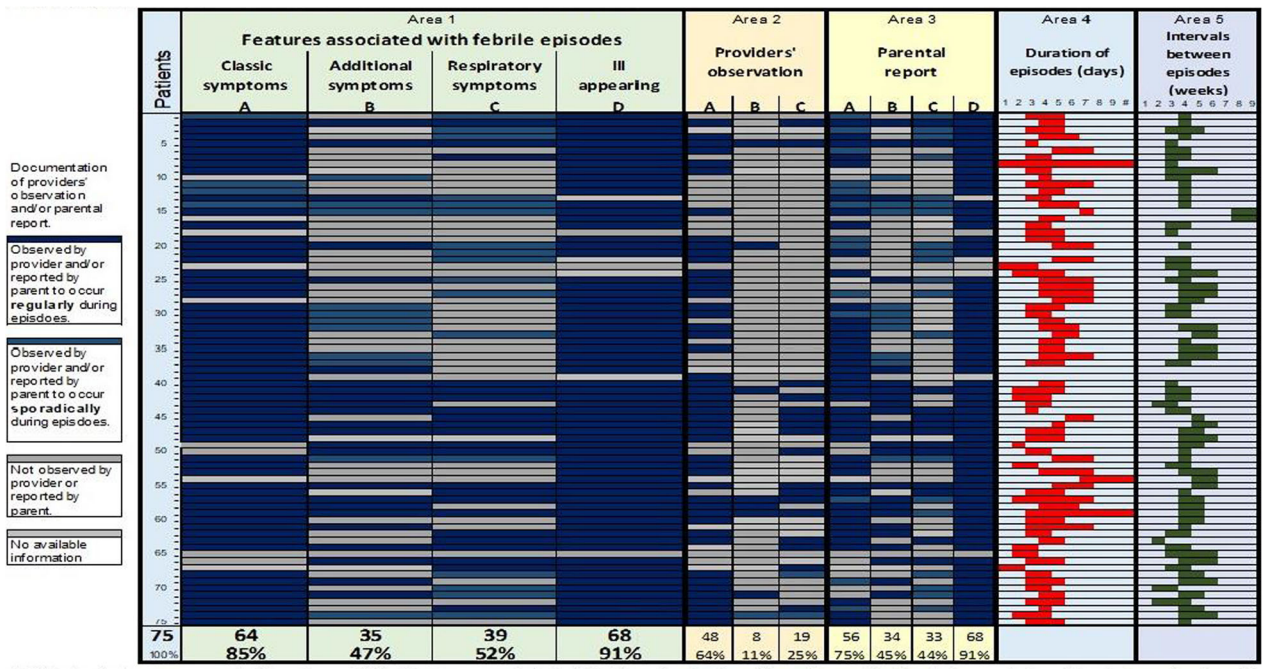
(+)M04.8: Other autoinflammatory syndromes: Blau syndrome, Deficiency of interleukin 1 receptor antagonist [DIRA], Majeed syndrome, PFAPA, Pyogenic arthritis, pyoderma gangrenosum, and acne syndrome

Table: Clinical signs and symptoms associated with febrile episodes, as documented in medical charts

Classic Symptoms [A]	Additional symptoms [B]	Respiratory symptoms [C]	Ill appearing [D]
Aphthous stomatitis	Headache	Cough	"decreased energy", "fatigued", "irritable", "cranky", "fussy", "tired", "quite", "cuddley", "clingy", "whiny", "decreased appetite"
Pharyngitis	Abdominal pain	Congestion	
Adenitis	Arthralgia	Runny nose	
	Rash	Dyspnea / tachypne	

Table 2: Clinical features of febrile episodes in children diagnosed with PFAPA



Individual patients are represented in each row. Clinical features associated with febrile episodes (area 1), are the combination of documented providers' observation (area 2), and/or parental report (area 3). Standardaized signs and symptoms are defined as (A) classic, (B) additional, (C) respiraotry, and (D) ill appearing [see table 1 for further details]. Episodes further characterized by their duration in days (area 4) and intervals between, in weeks (area 5).

(91) Submission ID#808074

Hepatitis B Core Antibody Positivity from Immunoglobulin Therapy

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Abstract/Case Report Text

Background: Immunoglobulin therapy is used to treat patients who are immunocompromised from primary immunodeficiency,

chemotherapy, and autoimmune diseases. Hepatitis B serologies are often checked in patients who are immunodeficient and prior to initiating immunosuppressive therapies. The Hepatitis B Core Ab is a sensitive marker for previous or current infection and a positive result may prompt anti-viral therapy. We present a case of passive Hep B Core Ab transfer with immunoglobulin therapy and highlight the importance of understanding this phenomenon to avoid inappropriate treatment and misinterpretation of serologies. Previous literature has also presented similar cases, but awareness is limited.
Case Presentation: A 38 year old Caucasian female with a history of common variable immunodeficiency complicated with granulomatous interstitial lung disease and liver disease (nodular regenerative hyperplasia), inflammatory bowel disease, immune thrombocytopenic purpura presented for follow-up in clinic. She was receiving weekly 12g subcutaneous immunoglobulin therapy. Her hepatitis B serology in 12/2015 demonstrated Anti-hep B core positivity. The patient also had elevated liver function tests at the time with alanine aminotransferase of 70 and aspartate aminotransferase of 57 and alkaline phosphatase of 464 so the hepatitis surface Ag was checked and was negative. She later had a liver biopsy for her transaminitis showing nodular regenerative hyperplasia. In 2/2016 her Anti-hep B core was rechecked and was negative. In 7/2019 there was possibility of placing patient on rituximab for her lung disease, her Anti hep B core was checked again and had converted back to positive. Her hep B surface antigen levels were consistently negative. Her hepatitis B DNA quant was also negative. Our patient illustrates immunoglobulin therapy may passively transfer hep B c Ab and can result in conversion of serologies without true hepatitis B infection.
Conclusion: Passive conversion of hepatitis B serologies should be considered in patients on immunoglobulin therapy. In patients with elevated liver enzymes and anti hep B core positivity a hepatitis surface antigen and HBV DNA can be checked to verify infection. Increased awareness of this occurrence can decrease inaccurate diagnosis and unnecessary therapies.

	11/27/19	11/19/2019	10/28/2019	7/15/2019	2/16/2016	12/17/2015
Hep B surface Ag		negative	negative	negative	negative	negative
Hep B surface Ab quant		411.57	393.22	300.36	233.76	324.21
Hep B core Ab total	positive	positive	positive	positive	negative	positive
IgG level Total	900	930	1051	739	810	822
Hepatitis B DNA quant	negative					

(92) Submission ID#808132

Characterization of the Clinical and Immunological Phenotype and Management of 157 Individuals with 56 Distinct Heterozygous NFKB1 Mutations

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Abstract/Case Report Text

Heterozygous mutations in NFKB1 are frequently identified among immunodeficient patients with highly variable clinical symptoms. In a world-wide collaborative effort, we characterized the clinical and cellular phenotype and the management of 251 of these patients harboring 106 distinct NFKB1 variants.

NFKB1 encodes the transcription factor precursor p105 which is processed to p50 (canonical pathway). Known pathogenic variants cause p50 haploinsufficiency (due to protein decay) or p105-skipping (with expression of p50-like forms). Most variants however are single amino acid changes with yet unknown effects. All sequence changes were assessed in silico for their probability of pathogenicity including 32 variants which were additionally tested in vitro. These analyses include the sub-cellular protein localization (microscopy), protein expression, stability and processing (Western blotting), transcription factor activity (reporter assay) and DNA-binding (EMSA) in HEK293T cells transfected with synthetic constructs. So far, 56 distinct variants in 157 individuals from 68 unrelated families indicated a pathogenic relevance.

The NFKB1-associated primary immunodeficiency disorder (PID) is characterized by hypogammaglobulinemia (88.9%), reduced switched memory B cells (60.3%), and respiratory (82.2%) and gastrointestinal (29.2%) infections. Incomplete penetrance (70%) and age-dependent severity of the clinical phenotypes were common. In addition, high frequencies of autoimmunity (57.4%), lymphoproliferation (52.4%), non-infectious enteropathy (23.1%), opportunistic infections (15·7%), autoinflammation (29.6%), and malignancy (15.7%) indicate immune dysregulation and multi-system involvement. Current treatment options include immunoglobulin replacement and immunosuppressive therapy. Our results suggest that hematopoietic stem cell transplantation and specific targeting of the NF-κB1 signalling pathways should be considered as alternative strategies in the future.

(93) Submission ID#808137

Changes in DNA Methylation Levels at the PI3KCD Locus in CVID patients

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Abstract/Case Report Text

Background. Common variable Immunodeficiency is the most common symptomatic primary immunodeficiency characterized by a loss of specific antibody production. Monogenetic causes have been identified in around 25-40% of patients. Lymphocyte differentiation is accompanied by demethylation processes. Previous work suggested impaired DNA demethylation during naïve-to-memory B cell transition in a cohort of 16 CVID patients. We investigated DNA methylation patterns at previously reported candidate sites at a set of genetically undefined CVID patients compared to healthy donors.

Methods. PBMCs of 19 patients and 19 healthy controls were sorted into four B-cells subsets (CD21low, naïve, IgM memory and switched memory) and four T-cell subsets (naïve CD4+, CD4+ EM, regulatory T cells [Tregs] and CD8+ effector memory RA [TEMRA]). Pyrosequencing was used to determine the methylation status at five previously reported CpG sites in the following genes: BCL2L1, PIK3CD, RPS6KB2, KCNC4, and CORO1B/PTPRCAP.

Results. Results from nineteen case-control pairs, matched for sex and age, demonstrated a significant change in the methylation level in naïve B cells in the CpG island of PI3KCD. For T cell subsets, we observed significant differences for KCNC4 (in Treg), CORO1B (in TEMRA cells) and RPS6KB2 (in CD4+EM). A late age-of-onset of CVID was associated with decreased methylation in PI3KCD in CD21low B cells. Autoimmunity was correlated with increased methylation levels in PI3KCD in IgM memory B cells and CORO1B in naïve B cells.

Conclusions. In CVID patients, significant differences were observed in PI3KCD (CpG1), which is mainly regulated at a transcriptional level. Our work adds evidence that epigenetic factors need to be taken into account to understand the etiology of CVID.

(94) Submission ID#808142

Infections In Secondary Immunodeficiency Patients Treated With Privigen Or Hizentra: A Retrospective US Administrative Claims Study In Patients With Haematological Malignancies

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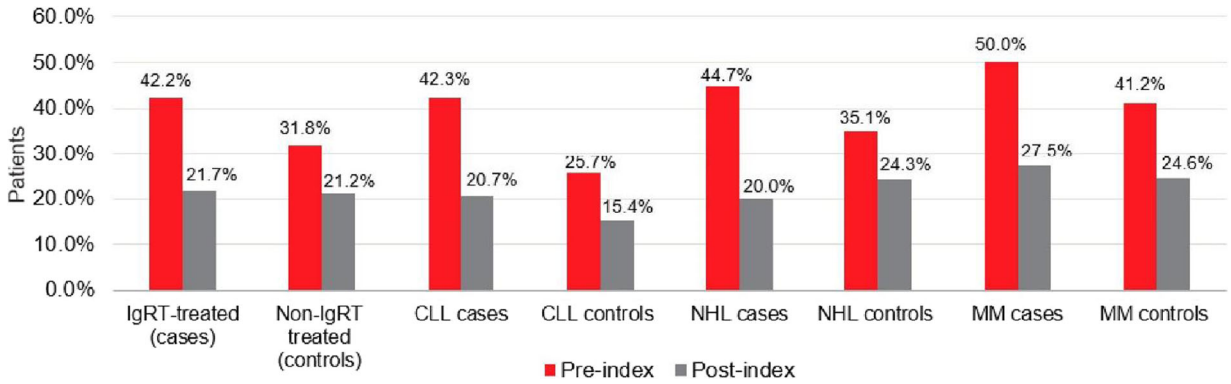
Abstract/Case Report Text

Introduction: Infections are a common outcome in patients with secondary immunodeficiency (SID)/hypogammaglobulinemia with underlying haematological malignancies. These infections are routinely treated with antibiotics and/or immunoglobulin replacement therapy (IgRT). This study evaluated the benefit of IgRT (Privigen® or Hizentra®, CSL Behring, King of Prussia, PA, USA) in terms of (a) occurrence and (b) change in frequency with IgRT of major/severe infections in patients with haematological malignancies and SID.

Methods: A retrospective database analysis was conducted using the IQVIA Real-World Data Adjudicated Claims – US Database (study period: January 2010–September 2018). Patients with an SID diagnosis were identified (diagnosis date) and stratified by underlying malignancy (chronic lymphocytic leukaemia, multiple myeloma and/or Non-Hodgkin's lymphoma). Those who had received Privigen® or Hizentra® (and no other IgRT) for 12-months following diagnosis date were defined as cases and age- and cancer subtype-matched patients who did not receive IgRT were defined as controls. Index date was date of IgRT initiation for cases while a pseudo-index date was assigned to controls (diagnosis date + days between diagnosis date and index date for matched case). Among cases and controls: (a) occurrence of major/severe bacterial infections (defined as inpatient hospitalisation with bacterial

infection and/or use of intravenous antibiotics in an outpatient setting) over the 12-month pre-index period (baseline) and 12 months post-index and (b) change in frequency of major/severe bacterial infections pre- and post-index were assessed.
Results: A total of 277 cases were matched to 1,019 controls. Mean age (57.2/56.6 years) and gender split (56.7%/55.5% male) were similar between cases and controls. At baseline, more cases had antibiotics claims (95.7% vs 87.0%, $p < 0.0001$) and more received cancer treatment (56.0% vs 45.9%, p=0.0031). Cases were more likely to have neutropenia, sinusitis or bronchitis (35.7% vs 27.1%, p=0.0049; 40.8% vs 26.9%, $p < 0.0001$; 25.3% vs 18.2%, p=0.0083) respectively, versus controls. Cases also had significantly higher pre-index healthcare costs ($219,149 vs $153,800; $p < 0.0001$). In the 12-month pre-index period, 88.4% of cases versus 72.9% of controls experienced any bacterial infection ($p < 0.0001$) and the proportion of patients who experienced ≥1 major/severe bacterial infection was 42.2% for cases versus 31.8% for controls (p=0.0011). The mean number of major/severe bacterial infections per patient in the 12-month pre-index period was 0.5 for cases versus 0.2 for controls (p=0.0014).
Evaluating post-index outcomes, 21.7% of cases versus 21.2% of controls had major/severe bacterial infections (Figure 1); the associated unadjusted odds ratio (OR) was not significant, suggesting IgRT had restored cases to a similar baseline infection risk as the controls. In a multivariate conditional logistic regression model adjusting for the significantly higher occurrence of risk factors in cases compared with controls in the pre-index period, cases were associated with a 31% lower adjusted odds of major/severe infections in the post-index period versus controls (OR=0.69; p=0.04).
Conclusions: Patients who required treatment with IgRT (Privigen®/Hizentra®) had previously experienced more major/severe bacterial infections than those not needing treatment with IgRT. However, IgRT was associated with a reduction in the risk-adjusted odds of major/severe bacterial infections compared with non-IgRT treated SID patients with haematological malignancies.

Figure 1. Percentage of patients with ≥1 major/severe bacterial infection pre- and post-index in cases or controls



(95) Submission ID#808148

Risk Factors For Major/Severe Infections in Secondary Immunodeficiency: A Retrospective US Administrative Claims Study In Patients With Haematological Malignancies

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Abstract/Case Report Text
Introduction: Real-world data are lacking as far as identifying patients with secondary immunodeficiency (SID)/hypogammaglobulinemia who may benefit most from interventions to protect them from potentially fatal infections. This study aimed to identify risk factors for major/severe infections in patients with SID with underlying haematological malignancies.
Methods: A retrospective database analysis was conducted using the IQVIA Real-World Data Adjudicated Claims – US Database (study period: January 2010–September 2018). Inclusion criteria were adults newly diagnosed with SID (first diagnosis termed the index date), with ≥12-months continuous health plan enrolment pre-index (baseline period) and a minimum of 3 months' data post-index (mean: 615 days), with chronic lymphocytic leukaemia, multiple myeloma and/or non-Hodgkin's lymphoma and without claims for any Ig therapy in the 12-month baseline period. Patient characteristics in the 12-month baseline period were assessed. Over the post-index period, antibiotic/antiviral use and frequency of infections were assessed. The frequency of major/severe infections was determined using diagnosis codes for bacterial, viral, fungal, parasitic, other or unspecified causal pathogen infections. Major/severe infections were defined as those requiring inpatient hospitalisation with an infection diagnosis code and/or use of intravenous (IV) antibiotics or IV antivirals in an outpatient setting. A multivariate Cox proportional hazards (PH) model evaluated baseline patient characteristics associated with risk of major/severe infections post-index.
Results: A total of 4,066 patients met the inclusion criteria. The mean age of patients was 57 years and 56.0% were male. In the 12-month baseline period: 75.6% of patients received cancer treatments and 87.0% of patients received antibiotics (27.1% IV antibiotics). A total of 79.5% of patients experienced any infection,

65.6% experienced ≥2 infections and 30.4% experienced major/severe infections. The mean number of infections over the baseline 12 months was 9.5 for any infection (at the unique diagnosis code level) and 0.7 for major/severe infections (unique hospitalisations with any infection diagnosis code and/or unique days with an outpatient IV antibiotic or IV antiviral). In the post-SID diagnosis period, 46.4% of patients had major/severe infections; of the major/severe infections, 34.0% were identified as bacterial, 11.6% were viral, 6.3% were fungal, while 48.2% did not have a causal pathogen specified. A total of 21.3% of patients experienced one severe/major infection and 10.5% experienced ≥4 severe/major infections (Figure 1). The mean annualised number of major/severe infections post-index was 1.5. Receiver operating characteristic (ROC) curve analysis to optimise sensitivity versus false positives in identifying those at risk of major/severe infections post-index identified a cut-off point of three bacterial infections in the baseline pre-index period as a potential optimal trigger to consider treatment to avoid major/severe infections post-index (Figure 2). The multivariate Cox PH analysis suggested that hospitalisations, infections (≥3), or antibiotic use in the 12-months pre-index (prior to SID diagnosis) were predictive of major/severe infections post-index (post-SID diagnosis) (all $p < 0.0001$).

Conclusion: Infections are common in patients with haematological malignancies and SID. Key baseline predictors for major/severe infections in patients with an SID diagnosis were a history of infections, hospitalisations or antibiotic use.

Figure 1. Percentage of patients who experienced major/severe infections in the post-SID diagnosis period, by number of infections

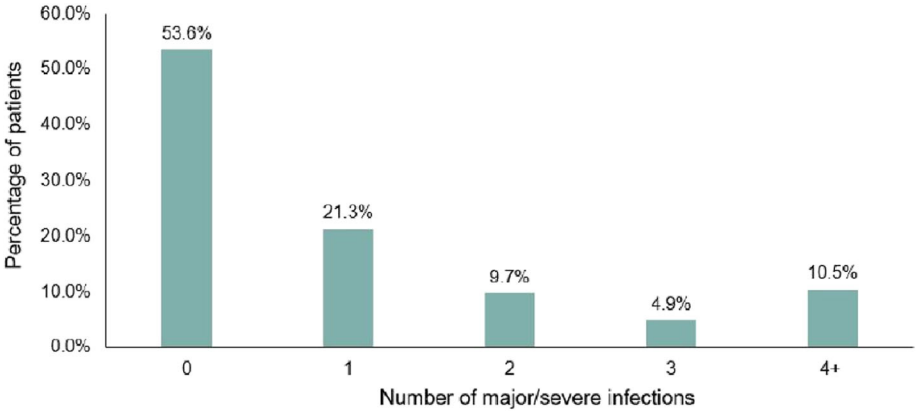
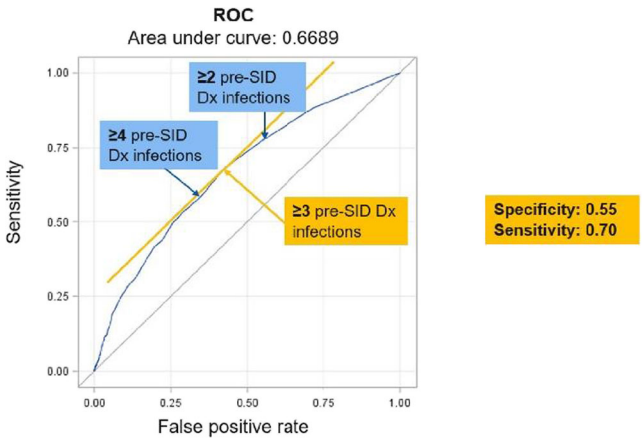


Figure 2. ROC curve for pre-SID diagnosis infections as a predictor of risk of major/severe infections post-SID diagnosis



(96) Submission ID#808315

Targeted Proteomics Panel for Clinical Diagnosis and Newborn Screening of Primary Immunodeficiencies from Dried Blood Spots

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Abstract/Case Report Text

Background: Tandem mass spectrometry (MS/MS) is a primary platform for many clinical and newborn screening laboratories. The application of MS/MS mainly focuses on the analysis of accumulated metabolites in plasma. Direct quantification of intracellular proteins would benefit diseases where such metabolites do not exist. Unfortunately, MS/MS detection is limited by the extremely low (e.g., pmol/L) protein concentrations in blood cells. Peptide immunoaffinity enrichment coupled to selected reaction monitoring (immuno-SRM) is a robust method for quantification of low

abundance proteins in complex matrices, including dried blood spots (DBS). In a study of 37 patients, immuno-SRM reliably identified Wiskott-Aldrich Syndrome (WAS) and X-linked agammaglobulinemia (XLA) patients using direct quantification of proteins responsible for disease (Front. Immunol., 2018). We further expanded our approach for X-linked Chronic Granulomatous Disease (X-CGD), ADA and Dock8 deficiency. Marker proteins representing platelets, NK cells, and T-Cells have also been analyzed to provide additional information about disease processes. These results demonstrate the utilization of immuno-SRM as a sensitive platform for multiplexed signature peptide quantification and its potential for PIDD newborn screening and clinical diagnosis from DBS.
Methods: Candidate peptides were selected based on MS/MS sensitivity and uniqueness in the proteome. Anti-peptide monoclonal antibodies (mAbs) were then generated for peptide enrichment from DBS. Blood from normal controls, XLA, WAS, XL-CGD, Dock8 and ADA deficiency patients was collected after consent on filter paper, dried, and stored at -20 °C. Proteins were extracted from DBS, digested with trypsin, and enriched using mAbs bound to magnetic beads. The enriched peptides were then eluted and analyzed with a Waters Xevo TQ-XS.
Results: A multiplexed Immuno-SRM panel has been generated for screening eight signature peptides representing five PIDD-specific and three cell-type specific proteins from DBS. Limits of detection and quantification were femtomoles of peptide, the assay showed a broad linear range, and intra-assay and inter-assay coefficients of variation were < 20%. In samples from 13 XLA, 8 WAS, 3 XL-CGD, 1 Dock8 and 1 ADA deficiency patients, signature peptides are significantly reduced relative to normal controls and patient identification had excellent agreement with clinical and molecular diagnosis. Also included in the multiplex panel are cell specific markers for platelets (CD42), T-Cells (CD3ε), and NK Cells (CD56). Diagnostic cutoffs for each peptide concentration have been established. In WAS patients, CD42 levels were significantly reduced consistent with characteristic thrombocytopenia. Immuno-SRM also has the ability demonstrate the effects of PIDD treatment. A WAS patient analyzed before and after bone marrow transplant showed normalized WAS protein and CD42 after treatment. Two ADA deficiency patients showed normal levels of ADA enzyme after RBC transfusion. Finally, a high-throughput (HT) Immuno-SRM method screens PIDD-specific peptides in a 2.5-minute runtime meeting high volume NBS workflow requirements. This HT method returned identical results to the standard Immuno-SRM PIDD panel.
Conclusions: The data herein demonstrate the feasibility of using Immuno-SRM as a broad clinical diagnostic for identifying and studying PIDD patients from easily collected and shipped DBS. Significantly, HT immuno-SRM workflows represent a promising potential option for NBS of PIDDs and other congenital disorders.

(97) Submission ID#808468

Artificial Thymic Organoids Reveal an Early Block in T Cell Differentiation In Patients Carrying Mutations In IKZF1

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Abstract/Case Report Text
We have previously used the artificial thymic organoid (ATO) system, based on the 3D aggregation and culture of a delta-like canonical Notch ligand 4-expressing stromal cell line (MS5-Dll4) with CD34+ cells, to study T cell differentiation from CD34+ cells obtained from patients carrying defects that are intrinsic to hematopoietic cells (RAG1-2, AK2, IL2RG) or that affect thymus development (DiGeorge syndrome). We now report results of in vitro T cell differentiation of CD34+ cells obtained from patients with either haploinsufficiency or dominant negative (DN) mutations of the IKZF1 gene. IKZF1 is an essential transcription factor expressed throughout hematopoiesis and involved in both lymphocyte and myeloid differentiation. Heterozygous germline mutations in IKZF1 give rise to distinct clinical phenotypes, depending on the nature of the mutation. In particular patients with IKZF1 haploinsufficiency present with common variable immunodeficiency (CVID) associated with B cell immune deficiency, B-ALL susceptibility, and autoimmune manifestations. No clinical T cell defects are evident among these patients, except for elevated naive and central memory CD3+CD8+ T cells. In contrast, patients carrying DN IKZF1 mutations present with combined immunodeficiency (CID) characterized by the presence of an increased proportion of naïve T cells, associated with defective generation of memory T cells, impaired T cell activation, signaling and proliferation, reduced T-helper (Th) polarization, and susceptibility to Pneumocystis pneumonia. Different mouse models of IKZF1 mutations have been developed, however their phenotype does not fully match what reported in patients, and in some models indicates a more severe defect in T cell development. To address these controversies and to gain novel insights into the effects of distinct IKZF1 mutations on human T cell development, we used the ATO system to analyze progression of T cell development from CD34+ cells obtained from one patient with IKZF1 haploinsufficiency and one patient with DN IKZF1 mutation.
Both patients showed a similar early block in T-cell differentiation at pre-T cell stage. However, the patient with IKZF1 haploinsufficiency showed a more pronounced leakiness, with a residual production of CD3+TCRab+ cells, which could account for the milder T-cell phenotype presented in this type of patients. Interestingly, the DN patient presented an increased accumulation of CD4-CD8b-CD8aa+ cells. These results show an unexpected role for IKZF1 in humans in early stages of T-cell differentiation and indicate IKZF1 as a necessary factor for the induction of CD8b expression in T cells.
Funding: This work was supported by the Division of Intramural Research, NIAID, NIH

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Characterization of the Immunological Phenotype in a Patient with Indeterminate Pediatric Acute Liver Failure (iPALF) and Aplastic Anemia (AA)

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Abstract/Case Report Text

Background: Pediatric acute liver failure without an identifiable cause (indeterminate PALF/iPALF) is associated with increased rates of liver transplant and mortality. Aplastic anemia (AA) may develop weeks after the diagnosis. The immunologic mechanisms that contribute to disease pathogenesis have not been clearly elucidated. We report detailed Immunophenotyping of a patient with iPALF/AA.

Case: A previously-healthy 7-year-old male was admitted for acute hepatitis presenting with jaundice and hepatosplenomegaly. Evaluation for infectious, toxic, metabolic, autoimmune, and rheumatologic disorders was negative. He was pan-lymphopenic (CD45+ALC 445 cells/μL) with an inverted CD4:CD8 ratio of 0.2 on admission.

Liver biopsy showed severe portal, interface, and lobular inflammation characterized by activated sinusoidal macrophages and perforin-expressing CD8+T-cells. Compared to a healthy control, the percentage and number of peripheral blood CD8+T-cells expressing perforin (20%v.94%, 85 v.153 cells/μL) and granzyme-A/B (15%v.90%, 66 v.147 cells/μL) was also increased, while percentages of perforin+ (96%) and granzyme-A/B+(98%) NK cells were normal. Bone marrow (BM) showed 75% cellularity with rare hemophagocytosis. Serum cytokine analysis demonstrated IL-18 4052 pg/ml, IL-18-binding-protein 23003 pg/ml, CXCL9 4436 pg/ml, and sIL-2Rα 7979 U/ml, consistent with smoldering hemophagocytic lymphohistiocytosis (HLH), but he did not meet HLH diagnostic criteria. Genetic sequencing did not identify pathogenic variants in 207 genes associated with primary immunodeficiencies.

He was diagnosed with iPALF and treated with three doses of anakinra and two weeks of ruxolitinib, followed by prednisone 1-2 mg/kg/day and intravenous immunoglobulin 1g/kg/month. Immunophenotyping performed after two months of therapy showed persistent inversion of the CD4:CD8 ratio with small expansions of CD3+CD4-CD8- cells and TCR··+T-cells. In the CD4+T-cell subset, there was a substantial paucity of naïve cells, with effector memory T cells (Tem) being more abundant than central memory T cells (Tcm). In the CD8+T-cell subset, the majority of CD45RO+cells were Tem with no detectable Tcm, and 49% of all CD3+T-cells were CD8+TEMRA. In both CD4+ and CD8+T-cell subsets, activated (HLA-DR+) and senescent (CD57+) subpopulations were increased, and the majority of cells expressed the exhaustion marker PD-1. The hepatic inflammatory infiltrate similarly reflected repetitive antigenic stimulation, with expansion of CD103+CD8+T-cells. Quantitative immunoglobulins and total memory B-cells and plasmablasts (CD19+CD27+) were normal for age. However, there were no circulating IgA-memory B-cells and a reduced number of IgG-switched memory B-cells (Table 1).

Given the severity of his phenotype and BM hypocellularity (5%), allogeneic HCT was performed using a matched-related-donor (10/10) with conditioning of Flu+Cy+Alemtuzumab. At D+30, he shows improved liver function but persistent pancytopenia, with transfusion-dependence for platelets.

Discussion: To our knowledge, this is the first description of detailed immunophenotyping in blood from a patient with iPALF/AA. Other studies have identified distinguishing hepatic infiltrates and cytokine/chemokine profiles that suggest excessive activation of cytotoxic T-lymphocytes and macrophages contribute to disease pathogenesis (Alonso et al, 2017). Our preliminary data supports this hypothesis and expands the spectrum of immune dysregulation in the T and B cell compartments, proposing a primary immune etiology. Immune dysregulation may be concordant with hyperinflammation and cytokine storm, the latter offering potential therapeutic targets. Early diagnosis and treatment of immune dysregulation may prevent development of AA.

Table 1. Immunophenotyping of a patient with iPALF and AA refractory to immunosuppressant therapy.

Immunologic Parameter	P1	Median of >36 Healthy Controls
CD45+/CD14- lymphocytes (cells/mm^3)	223	1670
CD3+ T cells (% lymphocytes, cells/mm^3)	81, 181	76, 1300
CD3+CD4+ T cells (% lymphocytes, cells/mm^3)	6, 14	47, 790
CD3+CD8+ T cells (% lymphocytes, cells/mm^3)	64, 142	25, 415
CD3+CD4-CD8- T cells (% lymphocytes)	11	3
TCR ··+ T cells (% CD3+T cells)	16	3
CD19+/CD20+ B cells (% lymphocytes, cells/mm^3)	4, 10	11, 160
CD16++/CD56+ NK cells (% lymphocytes, cells/mm^3)	13, 30	12, 180
Naive CD4+ T cells, CD4+CD45RA+ CD62L+CCR7+ (%CD4+CD45RA+)	0	99
Central Memory CD4+ T cells, CD4+CD45RO +CD62L+CCR7+ (%CD4+CD45RO+)	10	55
Effector Memory CD4+ T cells, CD4+CD45RO +CD62L-CCR7- (%CD4+CD45RO+)	36	15
TEMRA CD4+ T cells, CD4+CD45RA+ CD62L-CCR7- (%CD4+CD45RA+)	55	0.2
Naive CD8+ T cells, CD8+CD45RA+ CD62L+CCR7+ (%CD8+CD45RA+)	1	78
Central Memory CD8+ T cells, CD8+CD45RO+ CD62L+CCR7+ (%CD8+CD45RO+)	0	16
Effector Memory CD8+ T cells, CD8+CD45RO +CD62L-CCR7- (%CD8+CD45RO+)	83	48
TEMRA CD8+ T cells, CD8+CD45RA+CD62L-CCR7- (%CD8+CD45RA+)	87	10
Activated CD4+HLADR+ T cells (%CD4+)	36	4
Activated CD8+HLADR+ T cells (%CD8+)	63	8
Senescent CD4+CD57+ T cells (%CD4+)	44	7
Senescent CD8+CD57+ T cells (%CD8+)	68	27
Exhausted CD4+PD1+ T cells (%CD4+)	95	18
Exhausted CD8+PD1+ T cells (%CD8+)	70	24
Transitional B Cells, CD19+CD24++ CD38++ (%CD19+)	11	9
Naive B Cells, CD19+CD27-IgM+IgD+ (%CD19+)	90	71
Total Memory B cells and Plasmablasts, CD19+CD27+ (%CD19+)	10	30
IgM Memory B cells, CD19+CD27+IgM+IgD- (%CD19+CD27+)	27	18
IgA Memory B Cells, CD19+CD27+IgM-IgA+ (%CD19+CD27+)	0	18
IgG Memory B Cells, CD19+CD27+IgM-IgA+ (%CD19+CD27+)	36	26
Marginal Zone B Cells, CD19+CD27+ IgM+IgD+ (%CD19+CD27+)	55	30

(99) Submission ID#809417

Nodular Regenerative Hyperplasia in X-Linked Agammaglobulinemia: An Underestimated And Severe Complication

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Abstract/Case Report Text
Background: X-linked agammaglobulinemia (XLA) is one of the first inborn errors of immunity identified, with thousands of patients described to date. Infections originally dominated the clinical phenotype, but early diagnosis and immunoglobulin replacement allowed for long term survival as well as recognition of late-onset complications. Nodular regenerative hyperplasia (NRH) of the liver is a silent cause of non-cirrhotic portal hypertension. NRH underlying pathophysiology remains blurry and the disease has no specific treatment. NRH has been increasingly reported in primary immunodeficiency but data in XLA are very limited.
Objectives: To assess and characterize NRH in patients with XLA.
Methods: We retrospectively reviewed the medical records of all XLA patients referred to the NIH between 1994 and 2019. Hepatology evaluation and liver biopsies were performed when clinically indicated. Patients were stratified into NRH+ or NRH- groups, according to their NRH biopsy status (patients with no liver biopsies were classified as Unknown). Laboratory values are presented as medians. Fisher's exact test and Mann-Whitney test were used to compare categorical and continuous variables, respectively.
Results: Twenty-one XLA patient records were reviewed, with a median age at start of follow-up (f/u) of 17y and a median duration of f/u of 10 years. Eight patients underwent at least one liver biopsy of whom 6 (29% of NIH XLA cohort) were NRH+. The median age at NRH diagnosis was 20y (17-31). Among patients who had liver biopsies, alanine aminotransferase (ALT) levels were mildly elevated in all, while alkaline phosphatase (ALP) levels were only increased in NRH+ patients (p=0.04). Both NRH+ and NRH- groups had similar aspartate aminotransferase (AST) levels at baseline but higher values were observed at the end of f/u in the NRH+ group (85 vs. 32 U/L, p=0.04). Persistently low platelet count (< 100k/μL for more than 6 months), mildly to highly elevated hepatic venous pressure gradient (HVPG) and either hepatomegaly and/or splenomegaly were present in all NRH+ patients. In opposition, neither persistently low platelet counts, nor hepato- or splenomegaly were present in the two NRH- patients evaluated. HVPG was normal in the only NRH- patient tested. All-cause mortality was higher among NRH+ patients (5/6, 83%) than in the rest of the cohort (1/15, 7% among NRH- and Unknown patients, p=0.002).
Conclusions: Based on our retrospective analysis, NRH appears as an underreported, frequent and severe late-onset complication in XLA, which is highly associated with increased mortality. Persistent thrombocytopenia, elevated ALP, elevated HVPG, hepato- and/or splenomegaly were common in liver biopsy-proven XLA/NRH+ patients and distinguish them from XLA/NRH- patients. Based on NRH prevalence, severity, lack of specific treatment and poor outcome in XLA, immune-reconstitution (rather than IgG replacement and infectious prophylaxis) should be considered early in this population in order to prevent fatal long term complications.

(100) Submission ID#809505

Hypogammaglobulinemia and Lymphopenia in Barth Syndrome

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Abstract/Case Report Text
Introduction: Barth syndrome (BTHS) is an X-linked recessive disorder caused by a mutation in the tafazzin (TAZ) gene resulting in an inborn error of cardiolipin phospholipid metabolism (an important mitochondrial inner membrane lipid). It is commonly characterized by intermittent neutropenia and cardiac and skeletal myopathies. We present a case of BTHS with associated lymphopenia

and hypogammaglobulinemia, which has not been previously described in the literature.
Case report: A two-month old male, born full term with normal newborn screening, was first admitted for RSV bronchiolitis. At this time, patient underwent an echocardiogram given his older brother with hydrops had died hours after birth and on autopsy was found to have dilated cardiomyopathy (DCM). Patient was similarly noted to have DCM and thus had whole exome sequencing done that showed a hemizygous mutation in the TAZ gene (c.639G>A). This novel variant resulted in early termination of the protein (p.Trp213Ter) with concern for loss of function. In regard to patient's first year of life, he had frequent URI symptoms, 6 episodes of acute otitis media requiring tympanostomy tubes, but no documented pneumonias or other serious bacterial infections. Patient also had gross developmental delay, particularly motor, and feeding difficulties with persistent failure to thrive requiring G tube placement. His absolute neutrophil count ranged from 900-6800 cells/mm3 in the first year.
At age 13 months, patient was found to be in acute decompensated heart failure with concern for myocarditis (CK 15,313 U/L, Troponin I 3.006 ng/mL) as well as acute hypoxic respiratory failure with respiratory cultures growing Pseudomonas. He was incidentally found to have an IgG level of 188 mg/dL (normal for age 345-1213) and treated empirically with IVIG. When seen by immunology, further workup showed persistent B cell lymphopenia (absolute CD19 of 167-377/mm3). He also had a low initial NK cell count (78-84/mm3, later normal) with normal CD8 and CD4 T cell counts. Tetanus and Hib titers could not be assessed as he had recently received IVIG. His IgG trended up to 931 mg/dL a few days after initial IVIG and then subsequently dropped to 632 mg/dL, with a level of 241 mg/dL two weeks following initial dose. Workup for gastrointestinal or renal losses of immunoglobulin were negative. He also shortly after developed Enterobacter bacteremia. His IgG levels at this time continue to remain around 270 mg/dL. He subsequently required a heart transplant at age 14 months for his DCM. After transplant, he continued to improve from a cardiac standpoint, but his lymphopenia persisted and each time he was weaned off IVIG, his hypogammaglobulinemia persisted at 275-372 mg/dL thus requiring additional IVIG replacement over the course of the next 8 months. The remainder of immunoglobulins were normal initially, but the IgM slowly dropped over time to 31-33.8 mg/dL. Patient was started on weekly subcutaneous immunoglobulin replacement at 22 months, doing well clinically at age 24-month follow up.
Conclusion: Here we present a patient with BTHS, with a novel variant, who had B-cell lymphopenia as part of his presentation with persistent hypogammaglobulinemia requiring IVIG replacement.

(101) Submission ID#809545

Validation Of STAT1 Gain-Of-Function Mutation With Decreased pSTAT1 In A Patient With CMV Carditis, CMC, Recalcitrant Thrombocytopenia, M. Abscessus Pneumonia

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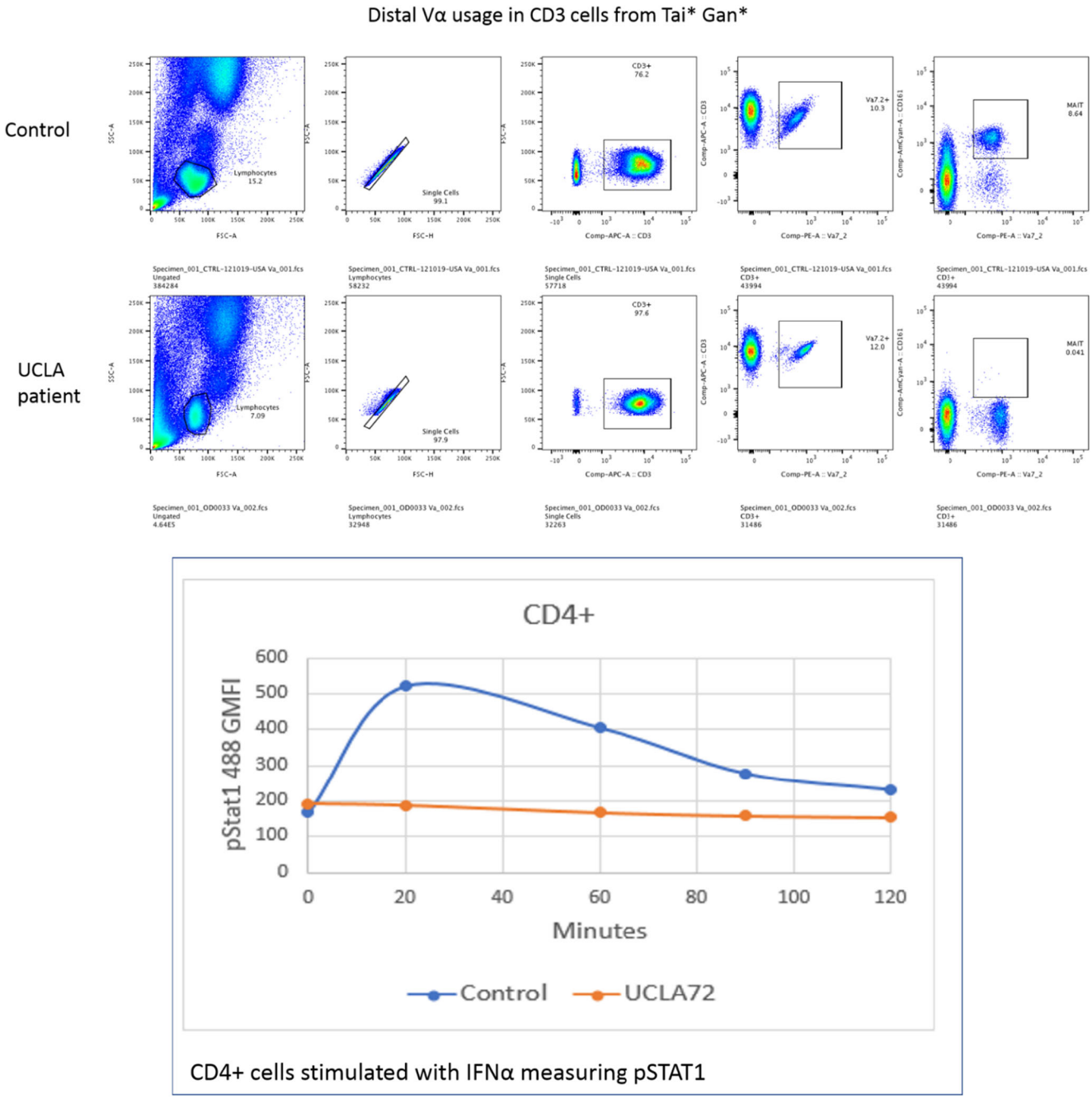
Abstract/Case Report Text
A 28-year-old female presented for combined immunodeficiency. At 2 years of age she was diagnosed with RAG1 hypomorphism and started on IVIG. As a child, she was hospitalized for pneumonias and cryptococcal meningitis. She suffered sinusitis, hepatitis, tooth abscess, CMV and herpes stomatitis. Later, she experienced recurrent cutaneous abscesses, UTIs, vaginal yeast infections, and hidradenitis. She twice hospitalized recently for pneumonias and diagnosed with Mycobacterium abscessus on bronchoscopy. She suffers onychomycosis, osteomyelitis and oral and esophageal candidiasis with odynophagia.
On exam, she had white plaques on tongue and buccal mucosa. She had hyperpigmented plaques on forehead and cheeks and thickened nails. Immune evaluation was significant for lymphopenia with ALC 770 and thrombocytopenia with platelets 102K. B cells were nearly absent (2 absolute count) and NK cells were low at 19 absolute count. IgE was absent, IgM 23 mg/dL, IgA 72 mg/dL and IgG 872mg/dL (on replacement). Her total CD3+ count was 791, CD4+ T cells were low at 17%, but CD8+ cells normal at 78%. The CD4+ T cells were mostly memory phenotype, which probably reflects lymphopenia-induced proliferation of a small number of clones. Her CD8+ T cells also had an elevated amount of memory cells for age, but still had presence of naive CD8+ T cells. As expected with perpetual lymphopenia-induced proliferation, there was evidence of terminal memory (TEMRA) in the CD8+ lineage. Proliferative responses of T cells were modest. CD4+ T cells did respond to pokeweed, but less to PHA and ConA. There were no antigen specific responses. TRECs were normal. ESR was mildly elevated at 31. Of note, her liver enzymes were elevated with alkaline phosphatase 556 and AST 116, presumably secondary to prolonged fluconazole use.
WES revealed a known pathogenic variant in STAT1 (NM_007315.3: c.537C>G (p.N179K)) as well as a heterozygous variant in RAG1 p.M355T. The STAT1 mutation is de novo and was previously published as a gain of function mutation. However, when we performed validation studies to evaluate CD4+ cells with stimulation to IFNa, the patient had decreased pSTAT1 as compared with control. Va7.2 analysis was performed to evaluate RAG1 defect and showed 12% of T cells with Va7.2 expression confirming that the RAG1 defect is not clinically significant.
She developed severe thrombocytopenia refractory to platelet transfusions and IVIG. She was started on Ruxolitinib which improved platelet counts. However, she presented with shortness of breath, persistent tachycardia and was found to have CMV carditis and hepatitis significant for echocardiogram with EF 23%. CMV PCR is improving with last check 815 IU/ml after 1 month of therapy with ganciclovir. We now are looking for evidence of SOCS1 to explain the decreased STAT1 phosphorylation.
Genetic testing is critical when evaluating a patient with immunodeficiency. Our patient demonstrates that genetic mutations cannot be taken at face value and should be evaluated and validated fully to optimize patient care.



(102) Submission ID#809613

Multiple Opportunistic Infections In A Hematopoietic Cell Transplant Patient Immunosuppressed With JAK and Complement Inhibitors

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Abstract/Case Report Text

Opportunistic infections (OI) are commonly seen in patients undergoing hematopoietic cell transplantation (HCT). Different strategies for antimicrobial prophylaxis are often employed in the transplant setting to reduce the likelihood of encountering infection. The predisposing risks for infections include the expected neutropenia and lymphopenia following conditioning, prolonged defects in cell-mediated and humoral immunity during the engraftment period, and iatrogenic immunosuppression by medications for graft versus host disease (GVHD).

We report the case of a 4-year-old male with acute lymphoblastic leukemia, which relapsed to chronic myelogenous leukemic blast crisis, and

failed a subsequent allogeneic HCT with central nervous system relapse. He was subjected to a second allogeneic HCT. His immediate post-second transplant course was complicated with skin and gut GVHD, and infection and/or reactivation of coronavirus, respiratory adenovirus, Epstein-Barr virus, and human herpesvirus 6. While the herpesviral infections were controlled with antivirals and rituximab, adenovirus C1 infection proceeded to involve the gastrointestinal tract, and proved persistent over several months despite use of cidofovir.

The patient's GVHD and transplant-associated thrombotic microangiopathy necessitated use of further immunosuppressants, including the complement protein C5-binding eculizumab (an inhibitor of formation of the terminal C5b-9 complex), ruxolitinib (a Janus kinase [JAK] 1/2 inhibitor) and low-dose interleukin-2. His clinical course was further complicated by Stenotrophomonas maltophilia gut colonization and subsequent bacteremia, as well as multiple Gram-positive bacteremia courses. At around day +190, there was a life-threatening pericarditis with pericardial effusion and respiratory distress, associated with Pneumocystis and Stenotrophomonas being isolated from bronchoalveolar lavage. This occurred despite the patient being on pentamidine prophylaxis. The patient eventually recovered on trimethoprim/sulfamethoxazole therapy.

We discuss the various risk factors potentially contributing to each OI in this illustrative case. In particular, complement and JAK inhibitor therapy are fairly new drugs approved for other indications, whose off-label use in transplant patients is increasing. Both have recently been associated with certain OI in the literature, as they are in this patient.

(103) Submission ID#809642

FAS Copy Number Variants Can Also Lead to ALPS, besides Germline and Somatic Variants

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Abstract/Case Report Text

Background: Autoimmune lymphoproliferative syndrome (ALPS) is characterized by chronic nonmalignant lymphadenopathy, splenomegaly, hepatomegaly, cytopenias, and other autoimmune manifestations. Typically, the biomarker profile of patients with ALPS includes elevated TCR αβ+ DNT cells, serum IgG, serum B12, serum IL10 and soluble FAS ligand (sFASL). HDL cholesterol can also be significantly low. ALPS is caused by lymphocyte accumulation due to defects in the FAS-mediated apoptosis signaling pathway. These defects cause resistance to physiological apoptosis in lymphocyte populations that results in chronic lymphoproliferation. The molecular defect underlying most ALPS etiologies is attributed to heterozygous germline or somatic (limited to DNT cell subpopulation) pathogenic single nucleotide variants (SNV) in FAS. We describe copy number variants (CNVs) at the FAS locus underlying ALPS in 3 unrelated families.

Methods: Through the Centralized Sequencing Initiative at at the National Institute of Allergy and Infectious Diseases (NIAID), patients undergo genomic workup to identify molecular defects contributing to clinical phenotypes of immune system disorders. All patients receive exome sequencing and a subset of patients also receive array-CGH analysis.

Patients and Results: We performed exome sequencing on 132 patients with a clinical diagnosis of ALPS. For 30 patients with no molecular defect through exome, we performed CNV analysis. In this cohort, we identified three patients with a copy number variant involving the FAS locus. All patients presented with splenomegaly and lymphadenopathy in childhood with ages of onset ranging from 8 months to 9 years old. All patients experienced anemia, autoimmune neutropenia, and thrombocytopenia. They had biomarker evidence showing elevated serum B12 levels, sFasL levels, and elevated αβ+DNT cell populations. They were found to have very low HDL cholesterol in early childhood ranging from 5-8mg/dL (38-55 mg/dL). All patients had negative family histories for lymphoproliferative disorders and immunodeficiency. These patients had clinical presentations and biomarker profiles similar to ALPS patients with germline and somatic FAS variants.

Patient 1: We detected a ~1.03 Mb copy number loss encompassing all of FAS. Parental studies were not performed.

Patient 2: We detected a ~1.004 Mb copy number loss encompassing all of FAS. Parental studies showed this to be maternally inherited. In addition, prior karyotype testing of the bone marrow showed the same deletion.

Patient 3: We detected a ~0.044 Mb copy number loss encompassing exons 7-9 of FAS. Parental studies were not performed.

These results are consistent with the pathogenic nature of copy number variant losses involving FAS. The mechanism of disease in these patients is consistent with haploinsufficiency. In family 2, the mother harboring the FAS deletion is unaffected. This is consistent with prior observation of reduced penetrance within a family in ALPS.

Conclusion: These three cases harbored causative deletions in FAS in the presence of biomarkers indicative of ALPS and negative results for germline and somatic genetic variant testing. These patients demonstrate that copy number variant analysis should be pursued if there is robust clinical and biomarker evidence of ALPS as it can lead to a molecular diagnosis and appropriate treatment when exome or next generation panel based FAS sequencing is inconclusive.

(104) Submission ID#809650

Lymphocytic Pneumonitis in a Thymoma Patient after Thymectomy

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Abstract/Case Report Text
RATIONALE: The thymus is essential for the development of T-cells. Patients with thymoma have decreased AIRE expression and have an abnormal thymic microenvironment where the negative selection of T-cells is compromised, resulting in a broad spectrum of autoimmune-mediated diseases. Besides myasthenia gravis, which is found in 15 to 20% of patients with thymoma, other autoimmune diseases have been reported including erythroblastopenia, systemic lupus erythematosus, inflammatory myopathies, thyroid disorders and Good's syndrome. Recent studies have described additional autoimmune conditions such as pneumonitis in thymoma patients. We identified a patient who developed chronic cough post-thymectomy and was found to have lymphocytic pneumonitis with associated autoantibodies against lung antigen KCNRG and lung immunopathology consistent with APECED pneumonitis, which implies a common pathogenic mechanism between these conditions.
METHODS: We describe a patient with thymoma who developed autoimmune pneumonitis associated with KCNRG autoantibodies and a characteristic pattern of immunopathology recently described in patients with monogenic disorder caused by primary AIRE deficiency (APECED) and secondary AIRE deficiencies (thymoma, RAG deficiency).
RESULTS: Patient is a 32-year-old male with no significant past medical history who was in good state of health until age 20 when he was diagnosed with and received treatment for guttate psoriasis (resolved with UV therapy) and alopecia areata. At age 30, he developed severe abdominal pain and weight loss. He had an abdominal CT performed that showed chronic pancreatitis and thymoma. One month later, the patient underwent thymectomy and subsequently, underwent ERCP and pancreas biopsy, revealing atrophic pancreatitis with negative staining for lgG and lgG4. At that time, he was started on pancreatic enzymes with improvement of abdominal symptoms. Following thymectomy, he developed persistent dry cough and recurrent symptoms of sinusitis which did not respond to several courses of oral antibiotics to treat his positive culture for Pseudomonas. He had a negative work up for vocal cord dysfunction and cystic fibrosis, and negative autoantibodies against IFN-gamma, IL-17A, and GM-CSF. For work up of chronic cough, the patient underwent CT imaging of the chest which revealed diffuse peri-bronchial thickening, mucus plugging, and tree-in-bud nodularity through most of his lungs. He underwent bronchoscopy with BAL which revealed normal bronchial mucosa and airway neutrophilia. Endobronchial biopsies showed basement membrane thickening and dramatic lymphocyte infiltration in intraepithelial and submucosal areas. His BAL cultures revealed Mycobacterium intracellulare/chimaera. Patient was also tested for autoantibodies against lung-specific bactericidal/permeability-increasing fold-containing B1 (BPIFB1) and the potassium channel regulator KCNRG that have been associated with the development of pneumonitis in patient with APECED, thymoma and RAG deficiency, and was found to have KCNRG-targeted autoantibodies.
CONCLUSIONS: Thymoma is a disease associated with secondary AIRE deficiency. This case illustrates common clinical, radiographic, histological, and autoantibody features in thymoma-associated and APECED-associated pneumonitis, indicating that disorders with primary and secondary AIRE deficiencies may have common pathogenetic mechanisms. BPIFB1 and KCNRG should be included in the autoantibody profile testing of patients with thymoma and lung disease. Immune suppression and antimycobacterial antibiotic treatment are planned.

(105) Submission ID#809688

Activated PI3K Delta Syndrome (APDS) in Two Peruvian Pediatric Patients

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Abstract/Case Report Text
Introduction/Background: Activated phosphoinositide 3-kinase δ (PI3Kδ) syndrome (APDS) is a primary immunodeficiency caused by a gain-of-function mutation in the PIK3CD gene that encodes the p110δ catalytic subunit of PI3Kδ. It is characterized by recurrent respiratory tract infections, lymphoproliferation, nodular mucosal lymphoid hyperplasia, enteropathy, EBV and/or CMV infection, reduced T cell function and high levels of IgM. There is not evidence of this disease in Peruvian patients.
Methods: A case series of two pediatric patients with APDS.
Results: The first patient is a girl of non-consanguineous parents. Family history shows four maternal uncles died at pediatric ages with unknown diagnosis. At the age of 2, she presented lymphadenopathy and fever being treated as cat scratch disease without improvement of symptoms. 8 months later, she was hospitalized due to anemia, mild hepatosplenomegaly, ascites and chronic diarrhea and diagnosed with gastrointestinal tuberculosis (TB). A hepatic biopsy only showed reactive hepatitis. However, the patient did not improve her symptoms despite anti TB treatment. 2 years later, she was hospitalized for lymphadenopathy, pancytopenia, chronic diarrhea, ascites and severe hepatosplenomegaly. CMV IgG was positive and lymph node biopsy revealed paracortical and follicular lymphoid hyperplasia due to EBV infection without neoplastic proliferation. Low CD4+ T and CD19+ B cells and high IgG levels were found (Table 1). At this time, it

was suggested the diagnosis of APDS which was confirmed by next generation sequencing (NGS) identifying a heterozygous mutation in the PIK3CD gene (c.3061G>A, p.Glu1021Lys). She was treated with sirolimus and IVIG for 2 years. The symptoms persisted despite treatment and died at the age of 6.

The second patient is a 6-year-old girl also of non-consanguineous parents. Family history includes eczema (father) and colorectal cancer (mother). She has had recurrent respiratory infections, chronic diarrhea and poor weight gain since 4 months old receiving symptomatic treatment only. At the age of 3, she was hospitalized for persistent pneumonia (Pseudomonas positive), lymphadenopathy and mild hepatosplenomegaly. A CT scan showed bilateral bronchiectasis and the sweat chloride test was negative. Based on this, a diagnosis of cystic fibrosis was made and treatment was started. However, a genetic study only showed heterozygous mutations in the CFTR gene (G551D and G542X). 1 year later, she presented a neck-located skin abscess. At the age of 5, she was hospitalized for complicated pneumonia, diarrhea, lymphadenopathy, ascites and severe hepatosplenomegaly. Multiple polyps in the duodenum and colon with lymphoid hyperplasia were detected, EBV IgM and IgG were positive and a lymph node biopsy showed paracortical hyperplasia without neoplastic proliferation. CD8+ T cells and IgM levels were increased (Table 1). A diagnosis of APDS was suspected and IVIG was started. NGS showed the same mutation as the first patient (c.3061G>A, p.Glu1021Lys).

Conclusion: APDS should be considered in patients with recurrent respiratory tract infections, lymphoproliferation, enteropathy and abnormal immunologic function without another explanation. NGS is a useful tool to identify these cases in low-income countries.

Acknowledgments: We thank Drs. Raif Geha and Janet Chou, Division of Immunology, Boston Children's Hospital, Harvard Medical School for the genetic diagnosis.

Table 1. Immunological profile of the patients

	Normal range	Patient 1	Patient 2
Lymphocyte subsets			
$CD3^+$, 10^3 cells/μL	0.9 – 4.5	1.14	**4.71**
$CD3^+CD4^+$, 10^3 cells/μL	0.5 – 2.4	**0.37**	0.95
$CD3^+CD8^+$, 10^3 cells/μL	0.3 – 1.6	0.67	**3.39**
$CD19^+$, 10^3 cells/μL	0.2 – 2.1	**0.17**	0.95
$CD3^-CD56^+$, 10^3 cells/μL	0.1 – 1.0	0.11	0.47
Immunoglobulins			
IgG, mg/dL	550 – 1020	**7218.3**	**1650**
IgM, mg/dL	46 – 150	119.8	**552**
IgA, mg/dL	54 - 153	**167.7**	**336**

(106) Submission ID#809845

Quantitative Computer-Aided Lung Informatics for Pathology Evaluation and Rating (CALIPER) assessment: A Newer Modality To Predict Progression in GLILD associated with CVID

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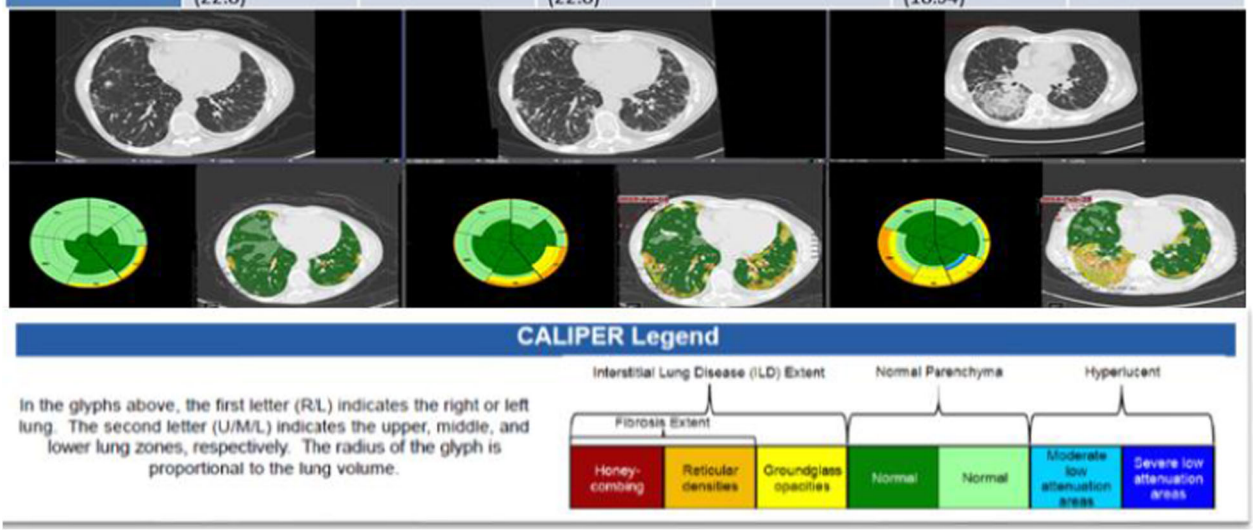
Abstract/Case Report Text

Background: Granulomatous-lymphocytic interstitial lung disease (GLILD) is an increasingly recognized pulmonary complication associated with common variable immunodeficiency (CVID) but the natural history and long term prognosis remains poorly defined. Imaging findings with computed tomography (CT) are heterogeneous and visual features do not consistently predict a patient's progression to fibrotic lung disease. Computer-Aided Lung Informatics for Pathology Evaluation and Rating (CALIPER) provides an objective analysis of lung parenchymal texture and quantifies the extent of normal lung, along with abnormal features such as honeycombing, reticular/consolidative and ground-glass opacity. This may be useful in CVID patients to monitor changes in character or extent of disease and may facilitate early intervention before the disease becomes more aggressive or advanced.

Case Description: Our patient is a 46-year-old non-smoking female with CVID who has been followed for her CVID and associated interstitial lung disease. For more than twenty years, she has had varying abnormalities found on chest CT and these appear consistent with GLILD. Specifically, she has had variable regions of mixed consolidation, ill-defined nodularity and septal thickening. The changing morphology and distribution made assessment of overall severity and extent of fibrosis versus parenchymal infiltration inconsistent. For clinical decision support we used CALIPER to analyze the current CT (2019) and compared CALIPER results for previous CT data. CALIPER provided a comprehensive analysis of the extent and characteristics of parenchymal features, and objectively determined normal and abnormal regions, some of which were not visually apparent. The CALIPER color overlay was able to highlight subtle regions of ground-glass opacity in areas that visually were regarded as uninvolved lung and quantify the extent of the reticular densities/consolidation over time. CALIPER does not differentiate reticulation from consolidation, does not detect nodularity or septal thickening, and CT imaging cannot distinguish inflammation from fibrosis. However, CALIPER has the power to quantitatively assess overall disease extent and demonstrate subtle abnormalities that would otherwise have been dismissed as normal, given relative sparing compared to other regions. CALIPER may also provide evidence for disease progression or therapeutic response that is not otherwise radiographically apparent.

Conclusion: CALIPER assessment may be a useful tool as an adjunct for a patient with GLILD to help quantify the extent and character of lung parenchymal involvement. This information may serve as an important guide for clinicians in the assessment of successful management and early intervention to prevent irreversible fibrosis.

	Oct 2019		July 2019		October 2014	
	Found	% predicted	Found	% predicted	Found	% predicted
Lung Volumes						
TLC	3.44 (4.78)	72%	3.41 (4.78)	71%	3.63 (4.82)	75%
VC	2.29 (3.43)	67%	2.19 (3.43)	64%	2.14 (3.22)	67%
RV	1.91 (2.63)	72%	1.22 (1.64)	75%	1.49 (1.55)	96%
Spirometry						
FVC	2.24 (3.43)	65%	2.18 (3.43)	63%	2.12 (3.22)	66%
FEV1	1.91 (2.77)	69%	1.97 (2.77)	71%	1.94 (2.71)	71%
FEV1/FVC	85.3 (81.2)	105%	90.6 (81.8)	112%	91 (76)	119%
Diffusion capacity						
DLCO_SB	12.6 (22.8)	56%	12.7 (22.8)	56%	13.62 (18.04)	76%



(107) Submission ID#809896

Immunodeficiency in patients with SGPL1 mutations Immunodeficiency in patients with SGPL1 mutations

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Abstract/Case Report Text

Objective: Evaluation of the immune system in two patients with recessive mutations in SGPL1 gene, encoding sphingosine-1-phosphate lyase (S1PL), was performed. Both patients were diagnosed with steroid-resistant nephrotic syndrome (SRNS), adrenal insufficiency, anemia, and hypothyroidism.

Methods: Flow cytometry was used for lymphocyte immunophenotyping and mitogen stimulation assay. The concentrations of total immunoglobulins and specific IgG titers to vaccines were also measured.

Results: Significant T cell lymphopenia was found in both patients, more pronounced in Patient 2. However, the patients have cleared respiratory viral infections reasonably well and have had no opportunistic infections. The T-cell numbers of Patient 2 have improved over time. Both patients had normal T cell proliferation to mitogens (Table 1). These findings may be explained by the known role of S1PL regulating lymphocyte trafficking (1). Both patients have a humoral deficiency with low B cell numbers and immunoglobulins titers, warranting IgG replacement therapy (Table 1). Despite this deficiency, patients have had very few bacterial infections and did respond to tetanus and diphtheria vaccines. The IgG levels have improved over time, perhaps partly secondary to adequate treatment of SRNS resulting in diminished proteinuria.

Patients had a trial of fingolimod without any beneficial changes in immune status. Both patients receive Pneumocystis jirovecii pneumonia prophylaxis with Sulfamethoxazole-Trimethoprim.

Conclusions: These results indicate that S1PL deficiency due to SGPL1 mutations is a syndromic primary immunodeficiency leading to profound lymphopenia and hypogammaglobulinemia. Our data emphasize the importance of sphingolipid metabolism for an efficient immune response and the need for more studies to delineate the exact mechanisms on how this happens in humans.

References

1. Lovric S, Goncalves S, Gee HY, Oskouian B, Srinivas H, Choi WI, Shril S, Ashraf S, Tan W, Rao J et al.Mutations in sphingosine-1-phosphate lyase cause nephrosis with ichthyosis and adrenal insufficiency.J Clin Invest. 2017 Mar 1;127(3):912-928.

Table 1. Immunological profile of the patients

Lymphocyte immunophenotyping	Values at 9-12 weeks of age			Values at 3rd year of age		
Reference ranges	Patient 1	Patient 2		Reference ranges	Patient 1	Patient 2	
WBC	5.0-19.5	6.0	5.1	5.0-19.5	2.1	5.3
Lymphocyte	2.5-16.5	1.4	0.9	2.5-16.5	0.4	0.9
CD3 (x10^9/L)	2.2-9.2	0.57	0.207	0.85-4.3	0.36	0.37
CD3+CD16/56+ (x10^9/L)	0.013-0.09	0.08	0.009	0.015-0.25	0.08	0.05
CD3-CD16/56+ (x10^9/L)	0.097-1.99	0.98	0.189	0.061-0.51	0.19	0.32
CD3+CD4+ (x10^9/L)	1.6-6.5	0.25	0.135	0.5-2.7	0.18	0.23
CD3+CD8+ (x10^9/L)	0.3-3.4	0.27	0.045	0.2-1.8	0.13	0.14
CD3+4+CD45RA+CD27+ (x10^9/L)	1.6-6.0	0.147	2.108	0.3-2.3	0.089	0.2
Naive CD3+4+CD45RA+CD27- (x10^9/L)	0-0.005	0.008	0.058	0-0.016	0	0.09
Terminally differentiated CD3+4+CD45RA-CD27+ (x10^9/L)	0.053-2.2	0.074	---	0.16-0.66	0.086	---
Central memory CD3+4+CD45RA-CD27- (x10^9/L)	0.02-0.021	0.021	---	0.004-0.089	0.005	---
Effector memory CD3+8+CD45RA+CD27+ (x10^9/L) Naive	0.29-1.65	0.225	0.03	0.053-1.1	0.114	0.12
CD3+8+CD45RA-CD27+ (x10^9/L)	0.01-0.19	0.004	---	0.004-0.064	0.008	---
Central memory CD3+8+ CD45RA-CD27-(x10^9/-L)	0.002-0.4	0.039	---	0.024-0.59	0.002	---
Effector memory CD3+8+CD45RA+ CD27-(x10^9/L)	0.013-0.82	0.039	---	0.025-0.53	0.005	---
Terminally diff CD19 (x10^9/L)	0.52-2.3	0.23	0.504	0.18-1.3	0.04	0.07
B cell- IgD-27+(x10^9/L)	0.01-0.17	0	0.012	0.02-0.22	0	0.01
Memory class, switched IgD+27-(x10^9/L)	0.62-2.12	0.21	0.351	0.28-1.33	0.04	0.05
Naïve IgD+27+(x10^9/L)	0.02-0.2	0	0.019	0.02-0.18	0	0.010
Memory, non-class switched IgA (g/L)	0.2-1.6	0.19	0.64	0.2-1.6	0.35	0.75
IgM (g/L)	0.2-1.6	0.82	0.52	0.2-1.6	0.26	0.2
IgG (g/L)	4-16	0.74	3.17	4-16	2.33	2.16
Specific IgG titers to vaccines		After first DTaP-IPV-Hib vaccination	First DTaP-IPV-Hib vaccination		After 4th DTaP-IPV-Hib vaccination	

(continued)

		done at age of 9 weeks	done at age of 14 weeks			
Tetanus Antitoxin (IU/ml)	>0.5-1.1	0.75	---	>0.5-1.1	---	0.83
Diphtheria Antitoxin (IU/ml)	>0.1	<0.010	---	>0.1	------	0.33
Mitogen proliferation assay	Reference ranges	At 4 months of age	At 2 months of age	---	---	---
PHA (Ki67/PCNA %)	58-98	80	83	---	---	---
ConA (Ki67/PCNA %)	62-94	76	86	---	---	---
PWM (Ki67/PCNA %)	15-55	64	68	---	---	---

(109) Submission ID#810163

The Influence of Sample Storage and Handling Conditions on Functional Complement Assessments using The Binding Site CH50 Assay on the Optilite Analyser

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Abstract/Case Report Text
Background: The CH50 assay is an important test for the functional assessment of the classical complement pathway. It acts as a screening test for classical cascade complement deficiencies, can monitor activation of the classical pathway and is a useful tool for assessing patients with autoimmune conditions. However, it is widely understood that appropriate sample handling and storage is pivotal to ensure accurate assessment of complement activity. Conversely, without a standardised approach to sample storage and handling, complement activation can occur ex-vivo leading to falsely abnormal results. Here we report on the stability of the classical complement cascade as measured by the CH50 liposome assay developed for use on the Optilite® analyser and comment on the optimal storage methods for reliable result generation.
Methods: Serum was collected from 61 healthy adult donors (self-certification absence of autoimmune conditions, fever or respiratory illness). For each sample, 6 replicates were performed on the day of collection (d0) and thereafter in duplicate with samples stored at either room temperature (~21°C ambient), 4°C or at -20°C (stability at -20°C was assessed after sequential freeze/thaw cycles). Sample stability was assessed based on CLSI guideline EP25-A with measurand drift used as the primary metric with an allowable error of +/-15%. All measurements were determined using the Optilite CH50 assay (The Binding Site Group Ltd, UK).
Results: The median value for samples at d0 was 65.17 U/ml (range 35.17 to 93.82 U/ml), with a median within-sample CV of 0.99% (range 0.18 to 6.2%). After 1d at room temperature there was a median -17% (range -33% to +2%; $p < 0.001$) change in CH50 activity. Subsequently there was a continued decrease in activity, which was time dependent; allowable error adjusted stability data indicated a median room temperature stability of 1d.
After 1d at 4°C there was a median -9% (range -44% to +3%; p=0.017) change in activity. After 2d -11% (range -48% to +8; p=0.006), 3d -13% (range -55% to +4%; $p < 0.001$), 4d -16% (-60% to +6%; $p < 0.001$) and after 7d -21% (-68% to +2% $p < 0.001$). A 4°C stability of 3d was determined from the median percentage reduction; total allowable error adjusted stability data indicated a 4°C stability of 5d.
Samples stored at -20°C following repeat freeze thawing saw a freeze/thaw cycle dependent decrease in CH50 activity. After 1 freeze/thaw cycle there was a median -9% (range -22% to +1%; p=0.018) change, 2 cycles -11% (range -26% to +2%; p=0.003), 3 cycles -13% (range -38% to +2%; $p < 0.001$), 4 cycles -23% (range -69% to -6%; $p < 0.001$) and after 5 cycles -25% (range -68% to -7%; $p < 0.001$). Allowable error adjusted stability data indicated a maximum of 3 freeze/thaw cycles.
Conclusion: Sample storage and handling can have a significant impact on functional complement assessments. Room temperature storage should be avoided unless samples will be analysed on the day of collection, 4°C storage is tolerable providing that assessment is within 3d; freezing samples at -20°C with limited freeze/thaw analysis would be optimal. However, further investigations into longer-term storage at -20°C and -80°C would be beneficial.

(110) Submission ID#810254

Association of GI status and Autoimmunity in CVID

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Abstract/Case Report Text
Background: Common variable immune deficiency (CVID) is a heterogeneous group of conditions affecting approximately 1:30,000 persons. The GI tract is the largest lymphoid organ and gastrointestinal (GI) complaints are frequent in CVID, leading in some to increased morbidity and higher mortality. Autoimmunity (AI) is similarly frequent in CVID patients. Previous studies have shown an association between AI conditions and CVID enteropathy. We investigated the relationship of GI status as a whole and autoimmunity among CVID patients in the USIDNET registry.

Methods: The United States Immunodeficiency Network (USIDNET) Patient Registry contains clinical data on patients with primary immune deficiencies. Data on 939 patients with a diagnosis of CVID were analyzed. Patients were considered GI+ if they reported any condition affecting the GI tract from mouth to anus excluding the spleen. 593 patients were identified as GI+ and 346 as GI-. Patients were further identified as having an autoimmune condition (AI+) or not (AI-).

Results: 1,075 autoimmune conditions were reported within the cohort, with some patients reporting multiple autoimmune diagnoses. Of 939 CVID patients, 39.5% (371) experienced at least one autoimmune condition. Within the GI subgroups, 45.0% (267) GI+ had at least one autoimmune condition, vs 30.0% (104) of the GI-. The most frequently reported autoimmune conditions over the entire cohort were ITP (119 in GI+ cohort, 50 in GI-), hypothyroidism (GI+ = 50; GI- =15), alopecia (GI+ = 20; GI- =9), psoriasis (GI+ = 20; GI- =9), Evans Syndrome (GI+ = 22; GI- =5), autoimmune anemia (GI+ = 21; GI- =7), Hashimoto's (GI+ = 18; GI- =8), celiac (GI+ = 23, GI- =0), rheumatoid arthritis (GI+ =17; GI- =4), and vitiligo (GI+ =13; GI- =6). The odds of being AI+ for GI+vs.GI- was 1.91 (95%CI: 1.44-2.52, p= < .0001). A sub-analysis indicated that ITP (OR: 1.49, 95%CI: 1.04-2.13, p=.03), hypothyroidism (OR: 2.03, 95%CI: 1.12-3.68, p=.02), and Evans syndrome (OR: 2.63, 95%CI: .99-7.00, p=.05) showed statistically significant increased odds in the GI+. Additional sub-analysis removing all GI autoimmune conditions from the AI+ group still resulted in statistically significant increased odds of autoimmunity 1.63 (95%CI: 1.23-2.16, p < .0007) if GI+.

Conclusions: GI disease is common in CVID affecting 63% of patients in our cohort. GI+ CVID patients have a higher frequency of autoimmune manifestations than those without GI complaints. The odds of ITP, hypothyroidism, and Evans syndrome all showed significantly increased odds in the GI+ group. The results of our study may have implications for both gastroenterologist and immunologist. Recurrent infections especially those of the sinopulmonary tract are often the trigger for CVID evaluation. Autoimmune and GI symptoms however may be the initial presentations of CVID and overlooked until other more recognizable manifestations evolve. The combination of GI issues and autoimmunity especially thrombocytopenia, Evans syndrome, and hypothyroidism should include CVID in the GI differential. For the immunologist, a CBC is standard in the work-up of CVID and may reveal autoimmune cytopenia. Evaluation for autoimmune disease and in particular hypothyroidism is not. Given our findings an initial immune work up specifically for thyroid disease may be indicated.

Association of GI status and Autoimmunity in CVID

Autoimmune Condition	GI+	GI-	Total
Thrombocytopenia (ITP)	119	50	169
Hypothyroidism	50	15	65
Alopecia	20	9	29
Psoriasis	20	9	29
Evans Syndrome	22	5	27
Hashimoto's	18	8	26
Celiac	23	0	23
Autoimmune anemia	21	7	28
Rheumatoid arthritis	17	4	21
Vitiligo	13	6	19
GI sub totals	323	113	436

(111) Submission ID#810400

Disseminated Histoplasmosis in an Autosomal Dominant Hyper-IgE Syndrome Patient

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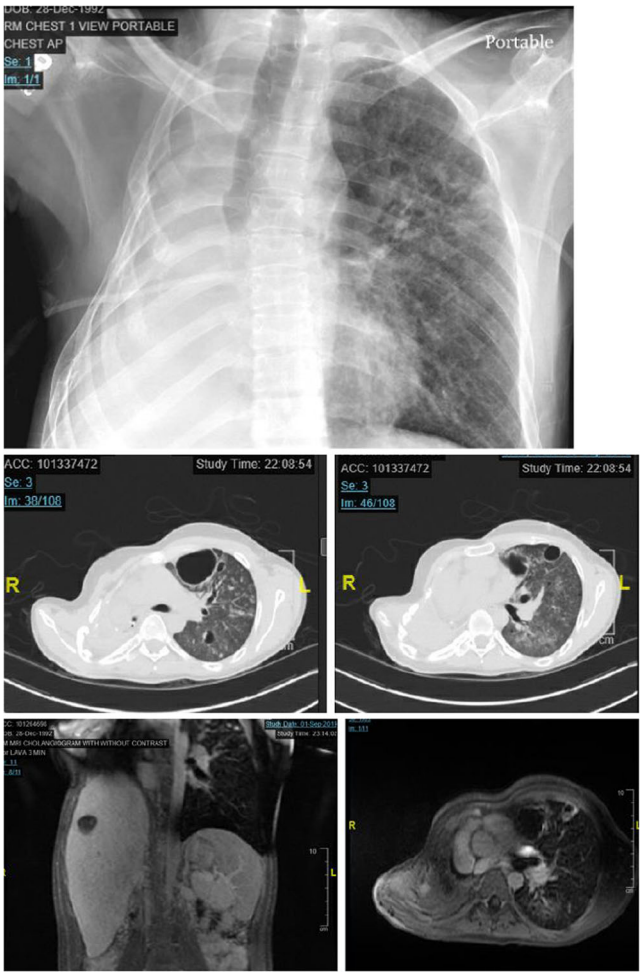
Abstract/Case Report Text

Introduction: Autosomal dominant hyper-IgE syndrome (AD-HIES), AKA Job's syndrome, is caused by dominant negative mutations in STAT3. Elevated IgE levels, low Th17 cell number, decreased CD4+ and CD8+ central memory T cells are the cardinal immunology features in patients, leading to increased susceptibility to bacterial and fungal infections. Histoplasmosis is an endemic and opportunistic fungal infection that causes impairment and symptoms in immunocompromised patients, most commonly in AIDS, but quite rarely in Job's syndrome.

Case presentation: A STAT3 mutation (c1144C>T) was previously identified in our 22-year old Hispanic Job's syndrome patient. He had history of atopic dermatitis, Hodgkin's lymphoma, skin cold abscess, and recurrent pneumonia, bronchiectasis, pneumatoceles with Staph aureus infections, lung Asperilloma, massive hemoptysis, as well as pulmonary embolism requiring right pneumonectomy. His management consists of prophylactic Bactrim DS, Voriconazole and monthly IVIG. Unfortunately, he lost insurance for about a year which prohibited him from specialty follow up and IVIG infusion. He was hospitalized five times in 2019 due to fatigue, shortness of breath and cough. His chest CT revealed bilateral bronchiectasis and cavitary lesions in the summer, which persist to date. BAL cytopathology was highly suspicious for fungal organism. He also had extremely elevated transaminase (ALT/AST 200-500s, AKP over 1600s) which prompted liver ultrasound showing liver cysts with cholestasis. Liver biopsy and culture was positive for Histoplasma capsulatum. Urine but not serum histoplasmosis antigen were positive. He was treated with intravenous vancomycin and cefepime for 30 days for clinical pneumonia, intravenous Ambisome B for 14 days for histoplasmosis and Gammunex during his last two admissions in our hospital. He is currently home on oral Itraconazole with improvement of symptoms. His potential risk factors for histoplasmosis infection include his travel to the endemic area Caracas (Venezuela), adoption of a dog early this year, and his inconsistent compliance to antifungal medication and IVIG infusion.

Discussion: We report an adult AD-HIES patient with disseminated histoplasmosis, identified by liver abscess culture. It was likely originated from the lung, as suggested by BAL cytopathology. Nine cases of histoplasmosis in AD-HIES patients have been reported in literature, eight of which primarily involved gastrointestinal tract. One case of disseminated histoplasmosis was found in a previously 33 month old child in his lung and blood. STAT3

is the transcriptional factor for many cytokines such as IL-17, responsible for T cell and neutrophil defense again fungal infection. STAT3 mutation leads to defect of neutrophil proliferation and chemotaxis to inflammatory site as well as production of antimicrobial peptides by respiratory epithelial cells. The poor tissue repair in the cavitary lesions and bacterial superinfection in patient's lung created a culture dish for fungal growth and dissemination. Traveling to the endemic area and patient's non-compliance to antifungal prophylactic treatment further increased the risk of histoplasmosis infection.



(112) Submission ID#810461

Interim Analysis of the Global Postauthorization Safety Study of Facilitated Subcutaneous Immunoglobulin Treatment in Patients With Primary Immunodeficiency Diseases

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Abstract/Case Report Text

Introduction/Background: fSCIG (immune globulin infusion 10% [human] with recombinant human hyaluronidase [rHuPH20]) is a subcutaneously administered immunoglobulin (IG) replacement therapy that is approved for patients who have primary immunodeficiency diseases (PID). fSCIG allows for enhanced IG distribution and absorption compared with conventional subcutaneous IG products and is associated with fewer systemic adverse events (AEs) than intravenous IG.

Objective: To acquire additional data on the long-term safety of fSCIG in patients who have PID.

Methods: This is an ongoing prospective, non-interventional, open-label, uncontrolled, multicenter, global postauthorization safety study initiated in the United States in November 2015 (NCT02593188). Patients aged ≥16 years with PID who have started fSCIG are followed according to standard clinical practice. Dosage regimen and treatment schedule are at the discretion of the treating physician. AEs are collected from enrollment to study completion/discontinuation. The presence of anti-rHuPH20 antibody titers is evaluated on a voluntary basis.

Results: As of May 2, 2019, enrollment was complete, with 264 patients enrolled at 32 US study sites and 81 patients still under follow-up. Most (79.2%) patients were female, with a mean (SD) age of 54.7 (15.7) years. No serious AEs (SAEs) related to fSCIG were reported. Infusions were self-administered at home (60.4%) or at the clinical site (39.6%), most commonly using 4-week infusion intervals (50.7%). The mean maximum IG infusion rate was 250.8 mL/h, and the mean IG dose was 400.8 mg/kg/4 weeks. The mean number of infusion sites used for administration was 1.9, and mean infusion duration was 3.0 hours. Most (98.1%) infusions were administered without a rate reduction, interruption, or discontinuation due to AEs. Twenty-seven patients experienced a causally related non-serious local AE (10.2%; 0.35 events/patient-year, 0.05 events per infusion), and 37 patients experienced a causally related non-serious systemic AE (14.0%, 0.72 events/patient-year, 0.10 events per infusion). Of the 194 patients with immunogenicity data, 14 (7.2%) had ≥1 positive binding antibody test to rHuPH20 (titers ≥1:160); no neutralizing rHuPH20 antibodies were detected.

Conclusion: This interim analysis of 264 patients with PID treated with fSCIG in routine clinical practice supports previous observations that fSCIG is well tolerated, with no reports of causally related SAEs or neutralizing anti-rHuPH20 antibodies.

(113) Submission ID#810555

IRAK4 Deficiency Presenting with Severe Viral Infections and Recurrent Kingella Kingae Sepsis

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Abstract/Case Report Text

We present the case of a 20 month old boy, the first child to his non-consanguineous parents of European descent. He first presented at 9 months of age with a cellulitis of his right fourth finger culture positive for Staphylococcus aureus which responded to a prolonged course of Flucloxacillin. At 12 months he presented with Norovirus positive gastroenteritis leading to a brief admission and slow resolution. The first of two severe episodes of oral stomatitis and respiratory distress occurred at 13 months of age. HSV1 was isolated from the oral lesions and blood culture during that admission was positive for Kingella kingae. No cardiac or bone involvement was identified. A more severe episode of oral stomatitis occurred two months later (age 16 months) swab positive for an Enterovirus (not typed). Due to airway compromise and rapid deterioration he was admitted to the Pediatric Intensive Care Unit. Again, Kingella kingae was cultured from blood cultures with no obvious focal systemic source.

The only notable clinical finding was rapid deterioration and, in retrospect, the absence of any significant recorded fever (< 38oC). CRP elevation was observed (max. 188 mg/L) and neutropenia was found with each of the more severe infectious presentations but recovered in the interval. Baseline immunological investigations were normal (lymphocyte subsets, naïve T cell populations, lymphocyte proliferation, serum immunoglobulins and vaccine responses). Serial measurement of circulating neutrophils did not identify a cyclical pattern and they were morphologically normal.

A panel of genes relevant to Primary Immunodeficiency (Invitae©) revealed a homozygous mutation in IRAK4 ((c.877C>T (p.Gln293*)) which leads to a premature stop codon. This is a known pathogenic mutation leading to disease and is most prevalent in the European population (allele frequency (gnomAD) = 0.0005). Prophylaxis with Sulfamethoxazole / Trimethoprim and Amoxicillin was commenced along with monthly IVIG. He has been well since diagnosis with no further severe infectious presentations. Functional testing is underway to assess in vitro host viral defence in our patient and potential novel mechanisms relevant to this rare innate immunodeficiency.

(114) Submission ID#810715

New Dangers Lurking: Diagnosis and Treatment of Fulminant Mulch Pneumonitis in Chronic Granulomatous Disease

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Abstract/Case Report Text

Chronic Granulomatous Disease (CGD) is an inherited primary immunodeficiency (PID) which results in both inflammatory response dysregulation and susceptibility to certain bacterial and fungal infections. Fungal pneumonias often have a subacute presentation. In contrast, fulminant mulch pneumonitis (FMP) secondary to aerosolized fungal and bacterial exposure can present as an acute respiratory emergency requiring prompt diagnosis and treatment in the face of high mortality.

Case studies will be presented on the five cases of FMP that were diagnosed and treated in 2019. Potential exposures were identified in four out of five cases: gardening exposure in one case and vaping exposure in three cases. All five were male, age range 14-45. Four were gp91 deficient, and one was p47-phox deficient. Historically, the vast majority of cases of FMP could be traced to a significant gardening exposure such as lawn mowing or spreading mulch. This was the first year that we saw patients with no identifiable gardening exposure in the setting of significant vaping exposure. With vaping at epidemic levels, especially among teenagers and young adults, it is important to consider that a vaping history is potentially a risk factor for FMP and counseling regarding the potential risks of vaping should be included in infection risk modification for all patients with CGD.

(116) Submission ID#810903

Rationale and Design of a Retrospective Chart Review Study on the Usage of CUVITRU in the United States: REToUCH Study

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Abstract/Case Report Text

Introduction/Background: Immunoglobulin (IG) replacement therapy is standard first-line treatment for most forms of primary immunodeficiency diseases (PID) with defective antibody production. CUVITRU (Immune Globulin Subcutaneous [Human], 20% Solution; Ig20Gly) is a highly concentrated formulation that enables subcutaneous infusion of IG in small volumes and reduces infusion times compared with less-concentrated products. Clinical trials have demonstrated the efficacy and tolerability of Ig20Gly in patients who have PID. While studies assessing real-world use of Ig20Gly are underway in other countries, there are currently no data available describing Ig20Gly use in real-world conditions in the United States.

Objective: To evaluate real-world patterns of Ig20Gly usage and administration in adult and pediatric patients in the United States who have PID and have been receiving Ig20Gly for at least 12 months.

Methods: REToUCh is a retrospective chart review study analyzing longitudinal patient chart data collected from two centers in the United States. Patients who are aged ≥2 years, have PID, and are incident Ig20Gly users who meet all inclusion criteria, with data for initial Ig20Gly infusion (index date) and for infusions occurring during the observation period at 6 and 12 months post-index, will be included in the analysis. Patients currently enrolled in another study or who had any treatment interruptions affecting >2 doses of Ig20Gly within the 12-month observation period prior to identification are being excluded. The planned enrollment size is 60 patients. Patient demographics, medical and PID treatment histories, reasons for selecting or switching to Ig20Gly, Ig20Gly infusion parameters (dosing and schedule; infusion rate, volume, and site; premedications; needle characteristics; administration setting; etc.), tolerability (defined by the incidence of local or systemic events and/or rate of discontinuation, interruption or reduction of infusion rate), and patterns of use at index date and 6 and 12 months post-index will be assessed.

Results: The REToUCH study is ongoing; real-world Ig20Gly tolerability and patterns of usage and administration in enrolled patients will be available for presentation during the congress.

Conclusion: The REToUCH study is expected to provide a detailed and complete description of Ig20Gly usage, tolerability, and safety under real-world conditions in patients who have PID in the United States.

(117) Submission ID#811047

Normal Mesenchymal Cells Can Restore Thymic Tissue Expansion In The Setting of 22q11.2 Deletion Syndrome

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Abstract/Case Report Text

BACKGROUND. 22q11.2 deletion syndrome (22q11.2del) is the most common human microdeletion disorder known, affecting 1/4000 individuals. Patients with 22q11.2del have variable congenital malformations, including a thymic hypoplasia that results in a T cell lymphopenia. Some patients will have a T-B+NK+ phenotype, leading to a severe combined immunodeficiency (SCID). The molecular cause of the thymic hypoplasia is unknown. Candidate cell populations responsible for thymic tissue expansion include neural crest-derived mesenchymal cells, thymic epithelial cells (TECs), and endothelial cells.

OBJECTIVE. To determine whether the hypoplastic thymus resulting from 22q11.2 deletion syndrome can be regenerated.

METHODS. Mouse models of 22q11.2del are used to characterize the embryological changes during the formation of the thymus within the 3rd pharyngeal pouch. Hypoplastic thymic lobes, evident as early as e12-e13.5, are isolated and characterized by flow cytometry and compared with normal tissues. In addition, the various cell populations from normal and hypoplastic embryonic thymic lobes are sorted by flow cytometry and used in reaggregate thymic organ cultures (RTOC), using various combinations of cell subsets. RNA sequencing on mesenchymal cells and single cell RNA-seq are being undertaken to identify transcripts required for thymic tissue expansion.

RESULTS. Flow cytometric analyses of normal and hypoplastic thymic lobes from e13 embryos revealed that both tissues had similar percentages of mesenchymal, epithelial, and thymic progenitors. The major difference with the hypoplastic thymus was a reduced cellularity suggesting a growth/expansion defect. While normal lobes expanded in fetal thymic organ culture, the hypoplastic lobes disintegrated. In RTOC, replacing 22q11.2del derived mesenchymal cells with those from normal fetal lobes restored the tissue expansion of the hypoplastic lobes. RNA Seq and 10X single cell RNA is revealing key transcripts involved in thymic tissue expansion, and these will be presented.

CONCLUSION. The thymic hypoplasia resulting from 22q11.2del syndrome is caused by mesenchymal cell defects. Thymic tissue regeneration and thymopoiesis are restored by the addition of normal mesenchyme.

(118) Submission ID#811053

Serum IgG Profiling of Healthy Toddlers Reveals a Subgroup with Clinically Informative Reactivities to Pathogens and Autoantigens

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Abstract/Case Report Text

BACKGROUND. The antibody repertoire in an infant/toddler is shaped by the microbiome, along with infections, diverse environmental exposures, and vaccinations. Monitoring the specificity of such antibody responses in normal toddlers will provide indicators of disease susceptibility.

OBJECTIVE. To determine whether profiling of the serum antibody specificities in toddlers provides key insights into the development of the immune response to pathogens and autoantigens.

METHODS. The serum IgG and IgM antibody reactivities in 1- and 2-year-old toddlers was conducted using an antigen array comprising infectious agents, autoantigens, vaccine antigens, and allergens. The toddlers were stratified based on their antibody reactivity to these antigens using a normalized fluorescence intensity measure. Repeat profiling was performed at year 2 to reveal longitudinal changes in the IgG responses. Clinical information, along with DNA sequencing, and selected cytokine assays were used to establish an odds ratio for disease potential among the cohort.

RESULTS. Toddlers exhibited a stratification into low, moderate, and high IgG responder groups unconnected with total serum IgG levels. The high responder group had elevated IgG reactions to selected pathogens and autoantigens. This group, representing 17% of the cohort, had high odds ratios with gestational diabetes, age, and a family history of asthma. While all toddlers developed strong antibody responses to Measles-Mumps-Rubella vaccines (MMR), more variation was noted towards other vaccines. In infections to Molluscum contagiosum, the IgG serum levels were transient regardless of the responder group. The high responder group had DNA polymorphisms linked to enhanced immune responses that correlated with elevated cytokine levels and eczema and asthma.

CONCLUSION. A subset of normal "healthy" toddlers has a high potential for immune system abnormalities and autoimmunity based on higher serum antibody responses to pathogens and autoantigens, genetic polymorphisms, and elevated cytokine responses.

(120) Submission ID#811088

Expanding The Phenotype Of XMEN disease: MAGT1 deficiency presenting with neurodegenerative symptoms

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Abstract/Case Report Text

X-linked immunodeficiency with magnesium defect, Epstein-Barr virus (EBV) infection, and neoplasia (XMEN) disease is a rare primary

immunodeficiency and glycoprotein-selective congenital disorder of glycosylation (CDG) caused by loss-of-function (LOF) mutations in the magnesium transporter gene (MAGT1). Patients present with lymphadenopathy, EBV-associated B cell malignancies, cytopenias, liver disease, elevated serum creatine kinase, splenomegaly, CD4 T-cell lymphopenia, and dysgammaglobulinemia. MAGT1 is part of the oligosaccharide transferase (OST) complex which is involved in asparagine-linked glycosylation of specific proteins. While mutations in other genes in this complex have been associated with non-syndromic intellectual disability (ID), the vast majority of XMEN patients have normal development. We report on 2 adult males with XMEN disease and normal developmental histories who developed progressive neurodegeneration as young adults, indicating a yet uncharacterized link between MAGT1 and central nervous system (CNS) degeneration.

Case 1: A 32-year-old male developed progressively worsening mania, dysarthria, recurrent falls, and ataxia at the age of 26 years. He had a past medical history of inflammatory liver disease and basal ganglia calcifications. His family history was remarkable for basal ganglia calcifications in his otherwise healthy mother and B-cell lymphomas in his brother and maternal uncle. Brain imaging showed bilateral frontal, subcortical and cerebellar calcifications as well as extensive cortical atrophy. Laboratory and whole exome sequencing (WES) did not reveal an explanation for his neurodegeneration or apparently familial basal ganglia calcifications. Surprisingly, he was hemizygous for a mutation in MAGT1 that results in a premature stop codon leading to nonsense mediated decay (c.414C>A; p.Tyr138X).

Case 2: A 33-year-old male with a past medical history of recurrent lymphadenopathy in childhood and complicated mononucleosis developed progressive gait and balance abnormalities, frequent falls, behavioral changes, seizure-like episodes, and cognitive decline at around the age of 20 years. Brain MRI showed cortical, cerebellar, and spinal cord atrophy as well as multifocal white matter lesions. Extensive evaluation for secondary causes of neurodegeneration was unrevealing. Genetic testing showed a LOF mutation in the MAGT1 gene (c.444dupT; p.Ala149Cysfs*6). Both patients had decreased NKG2D surface expression on CD8+ T and NK cells, expanded B cells, and chronic EBV viremia as determined by whole blood PCR. CSF PCR for EBV was negative on both patients. Both cases demonstrated abnormal carbohydrate deficient transferrin pattern, indicating a Type I CDG.

To our knowledge, these are the first cases of XMEN disease presenting with a progressive neurodegenerative phenotype. Further studies are needed to elucidate the basis of neurodegeneration and the role of MAGT1 in the central nervous system.

(121) Submission ID#811104

A Case Of Disseminated Cryptococcosis Gattii Due To Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) Autoantibodies

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Abstract/Case Report Text

Background: Granulocyte-macrophage colony-stimulating factor (GM-CSF) plays a critical role in macrophage and dendritic cell maturation and host defense against fungus. Autoantibodies to GM-CSF are associated with susceptibility to Cryptococcus and Nocardia infections as well as pulmonary alveolar proteinosis (PAP) in otherwise healthy individuals. We report a case of a 23-year-old previously healthy female who presented with cryptococcal meningitis and was found to have autoantibodies against GM-CSF.

Case Presentation: 6 weeks prior to admission, our previously healthy 23-year-old Taiwanese female developed a headache associated with tinnitus and visual changes. The headache worsened over the next few weeks and she developed photophobia, phonophobia, and severe nausea/vomiting. At presentation, her exam was notable for papilledema, bilateral CN VI palsy and right foot & left hand paresthesia. MRI Brain showed ring-enhancing lesions in the anterior frontal lobe, caudate head, and the inferior globus pallidus. She underwent a diagnostic and therapeutic LP. Opening pressure was elevated at 60 and CSF studies were notable for low glucose, elevated protein, pleiocytosis (94% lymphocytes) and positive cryptococcal antigen. CSF culture grew Cryptococcus gattii. CT Chest revealed a right upper lobe and a left lower lobe nodule.

Workup: CBC with Diff was unremarkable. HIV was negative. Lymphocyte subsets were unremarkable with only mildly decreased NK cells, normal immunoglobulin panel including IgE, protective titers to tetanus, diphtheria, and PPSV23. Targeted genetic sequencing did not identify any known mutations in primary immunodeficiency. Notably, Anti-GMCSF autoantibodies were detected by ELISA and were able to neutralize GM-CSF phosphorylation of STAT5 detected by flow cytometry. Autoantibodies to IFN-γ were not detected.

Management: Patient was initiated on a 6-week course of liposomal Amphotericin B and Flucytosine. Her CSF cultures were cleared of Cryptococcus after 20 days of treatment, but her hospital course was complicated by persistently symptomatic intracranial hypertension, worsening pleiocytosis, and elevated cytokine levels in the CSF, all of which were consistent with post-infectious inflammatory syndrome (PIIRS). She received therapeutic LPs 2-7x/week until subsequent ventriculoperitoneal shunt placement. Concurrently, methylprednisolone was administered for 7 days with a gradual prednisone taper. These interventions led to improvements in her symptoms, including diplopia, and reduction in opening pressures and inflammatory markers in the CSF. Lifelong fluconazole prophylaxis was recommended. From a pulmonary standpoint, she remained asymptomatic without signs of PAP and has had normal pulmonary function tests (normal DLCO) and stable chest imaging.

Conclusion: In otherwise healthy HIV-negative patients presenting with extrapulmonary Cryptococcus or Nocardia infections, autoantibodies to GM-CSF should be suspected and testing for functional autoantibodies to GM-CSF (and IFN-γ) should be sent, as

genetic testing will not pick up this disease entity. Genetic testing should be considered to rule out GATA2 deficiency and X-linked CD40L deficiency. Idiopathic CD4 lymphopenia can be ruled out with lymphocyte enumeration. Immediate treatment of cryptococcosis is not necessarily different from patients without GM-CSF autoantibodies. Long-term prophylaxis (fluconazole if presenting with Cryptococcus; Trimethoprim-sulfamethoxazole if with Nocardia) is likely warranted in addition to monitoring for the development of PAP. Recognizing PIIRS in patients with cryptococcal meningitis and management with corticosteroids are critical steps.

(122) Submission ID#811123

NF-κB-Driven Innate and Adaptive Immune Dysregulation Underlies Complications of Common Variable Immunodeficiency

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Abstract/Case Report Text

Background: Non-infectious complications cause most morbidity and mortality in common variable immunodeficiency (CVID). CVID with complications (CVIDc) is defined by elevated T helper 1 (Th1) responses attributed to increased circulating microbial products resulting from mucosal IgA deficiency. However, complications do not uniformly occur in those with IgA deficiency.

Objective: We tested whether CVIDc occurs preferentially in those with hyper-responsiveness to microbial stimuli, manifested by elevated NF-κB-driven cytokines and resultant Th1 responses in CVID patients with increased circulating microbial products.

Methods: We applied unbiased high-throughput seromics and mass cytometry, cellular and molecular biology approaches, and clinical record review in a 78 subject CVID cohort.

Results: CVIDc was defined by increased NF-κB-driven cytokines that promote Th1 immunity in blood in association with elevated soluble CD14, a marker of circulating microbial products, and elevated TNF production by peripheral blood mononuclear cells stimulated with lipopolysaccharide. This cytokine upsurge was associated with mutation of full-length NFKB1 p105 gene product (1375delT) but not mutations that also involved the NFKB1 p50 product involved in transactivation. Cytokine elevation corresponded with increased CD14+CD16- monocytes expressing higher CD86 and HLA-DR and more central and effector memory CD4+ T cells, T cell chemoattractants, and T cell-predominant tissue pathology. Those with granulomatous or neutrophil-predominant, rather than T cell, pathology had the highest TNF. TNF antagonism improved neutrophilic gastritis in CVID with NFKB1 1375delT after T cell targeted therapy failed.

Conclusion: NF-κB dysfunction underlies Th1 immunopathology and TNF-associated innate inflammation in CVIDc. Both forms of NF-κB immune dysregulation may divergently shape CVID immunopathology.

(124) Submission ID#811150

Daratumumab (anti-CD38) For Treatment of Disseminated Nontuberculous Mycobacteria in a Patient with Anti-IFN-γ Autoantibodies

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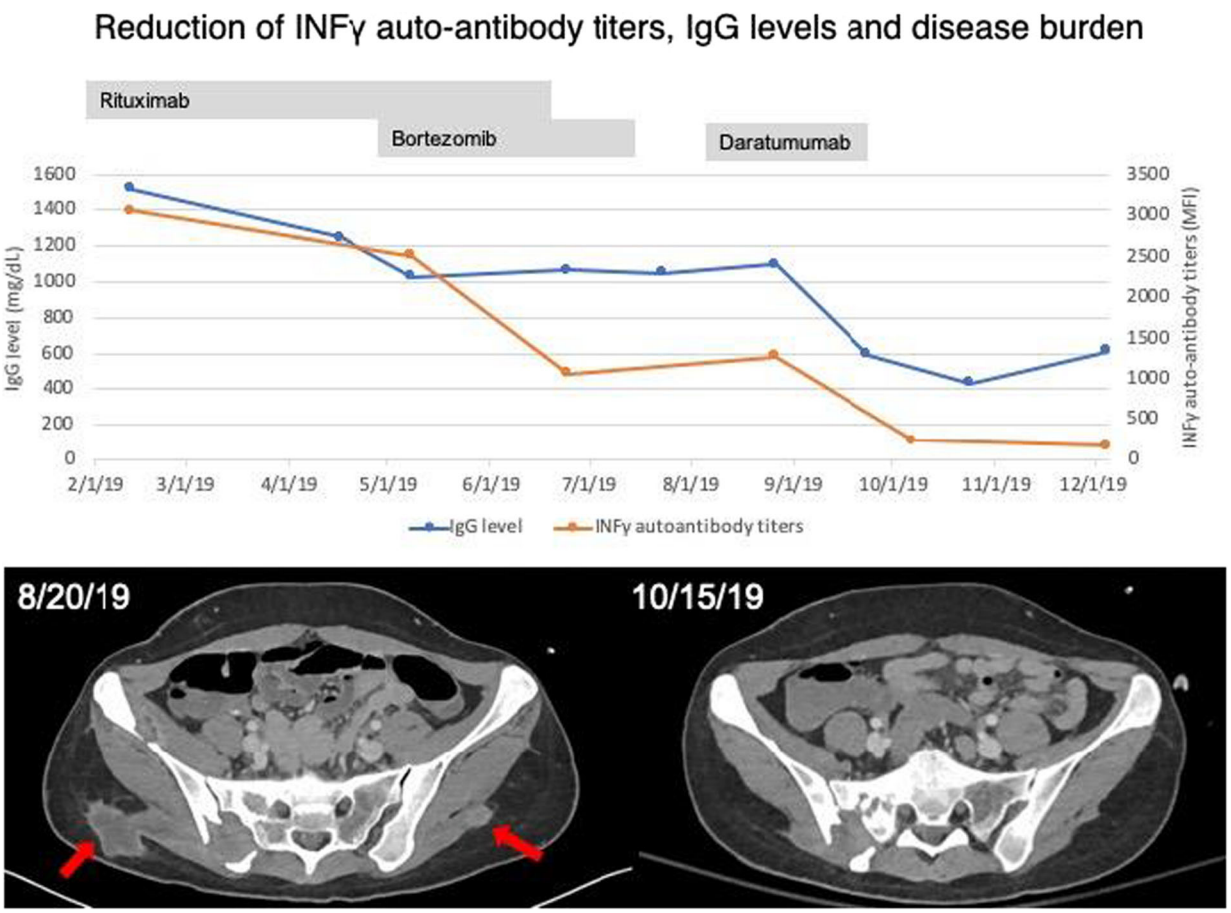
Abstract/Case Report Text

Introduction: Patients with autoantibodies to IFN-γ develop severe and progressive infections with intracellular pathogens, despite aggressive antimicrobial treatment. We describe the use of daratumumab (anti-CD38, targeting plasma cells) in a patient with autoantibodies to IFN-γ and progressive disseminated Mycobacterium avium infection. She had progressive disease despite treatment with multi-drug antimycobacterials rituximab, and bortezomib.

Methods: Clinical symptoms, total CD19/CD20, anti-IFN-γ autoantibody titers, and specific imaging were obtained before and after treatment with daratumumab. Anti-IFN-γ autoantibody titers were determined by serial 10-fold dilutions of plasma and measuring anti-IFN-γ autoantibody levels by a particle-based technique as previously described.

Results: A 31-year-old Filipino woman had progressive disseminated M. avium with extensive bone and soft tissue involvement (calvarium, ribs, bilateral arm soft tissue, paraspinal muscles, bilateral glutei, left inferior pubic ramus, bilateral iliac bones, sacrum, and bilateral humeri) and a tracheo-esophageal fistula. She received bedaquiline, azithromycin, ethambutol, tedizolid, moxifloxacin, clofazimine and meropenem as well as rituximab 1g once monthly for 5 months. Despite these she had progression of clinical and radiographic disease. Bortezomib 1.3 mg/m2 twice weekly for 8 weeks was added, but discontinued for AST and ALT elevations. Rituximab was continued to maintain CD20 numbers undetectable but clinical and radiographic disease progressed. While on rituximab, total IgG level and anti-IFN-γ autoantibody levels decreased from 1521mg/dL to 1069mg/dL and 3058 to 2504, respectively. While on bortezomib, total IgG levels remained stable (1031mg/dL to 1051mg/dL) and anti-IFN-γ autoantibody levels fell slightly (2504 to 1275). After starting daratumumab, there was clinical and radiographic improvement, with reduced pain and disappearance of multiple soft tissue lesions. IgG levels decreased from 1100mg/dL to 434mg/dL and anti-IFN-γ autoantibody levels decreased from 1275 to 157. Adverse effects of daratumumab were urticaria, pruritus and shortness of breath after the first infusion and aseptic meningitis after the 5th infusion.

Conclusions: Daratumumab resulted in clinical and radiographic improvement of disseminated M. avium in a patient with rituximab and bortezimib-refractory autoantibodies to IFN-γ. Daratumumab is another potentially effective therapeutic agent for anti-IFN-γ autoantibodies.



(125) Submission ID#811206

Supplemental NGS Method for Homologous Genes in Inborn Errors of Immunodeficiency Gene Panel (IEIGP)

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Abstract/Case Report Text

Next-generation sequencing (NGS) is now routinely used as a clinical diagnostic tool. However, regions of high sequence homology continue to be a major challenge for short-read technologies. Regions within IKBKG, NCF1, SBDS, C4A, C4B, CORO1A, FCGR3A, FCGR3B, PMS2, SLFN11, SLFN13, STAT5B, UNC93B1, and UPS18 are not available by standard NGS. We discuss strategies for analysis of these special regions.

We have developed a strategy for supplementing our disease targeted panels which are performed using capture chemistry and a standard reference file. The supplemental method uses gene specific long range amplicon and a special gene specific reference file for alignment. The genes of interest are separated from their homologous counterparts using specific long range amplification primers. Multiple amplicons may be pooled together and prepared for sequencing on an Illumina MiSeq instrument using TruSeq Nano DNA Library Prep. Bioinformatic analysis proceeds with a custom reference file in which non-specific regions of homology have been removed. This allows reads to be uniquely mapped despite significant homology; a requirement for variant calling. We prepared specific amplicon for several homologous gene targets including the IKBKG gene and the IKBKG pseudogene (IKBKGP1). Both amplicons were sequenced in separate reactions and were compared with the standard capture method. Variants which are not called in the standard-capture method due to poor mapping scores (non-uniquely mapped reads) are called in the amplicon method. In the capture method, the variants are visualized in the BAM as a mixture of gene and pseudogene, while gene and pseudogene variants are clearly separated and identified in the amplicon method.

Due to high variability in alignment, many homologous regions do not provide reliable Copy Number Variant (CNV) results and must be removed from CNV analysis. However in some situations, we are able to creatively leverage CNV analysis to identify alleles that mis-align to the pseudogene. The pathogenic NCF1 GT deletion in Exon 2 appears to resemble a copy number deletion event when present as reads from one allele mis-align to the NCF1B and NCF1C pseudogenes.

Complement genes, C4A and C4B, share alignment due to their high homology with each other. CNV analysis in normal samples represents four alleles rather than two alleles. CNV events may have a weak signal with no indication of which gene is affected. Variant frequencies from the capture and supplemental PCR analysis can be used in tandem with CNV analysis to detect events and may indicate which gene is affected.

We plan to include these strategies in our new Inborn Errors of Immunodeficiency Gene Panel (IEIGP) which will enable us to provide a more comprehensive analysis than is currently available.

(127) Submission ID#811256

Inflammatory Manifestations in Children with Chronic Granulomatous Disease

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Abstract/Case Report Text

Inflammatory manifestations (IM) are increasingly recognized as comorbidities among patients with Chronic Granulomatous Disease (CGD). The aim of the study is to describe the different inflammatory manifestations and the clinical and laboratory profile of patients with CGD who presented them.
Methods: Retrospective analysis of the electronic medical records of all CGD patients followed at the outpatient clinics of Pediatric Allergy and Immunology Unit at University of São Paulo Children's Hospital, between 2008 and 2019. Clinical, laboratory and histological data of patients with CGD patients who had IM were evaluated. Infectious or neoplastic causes were excluded.
Results: Thirty patients (28 male) with a median age of 13.1 years (1.2-18.2) in the last clinical follow-up were included. The diagnosis of CGD was confirmed by oxidation of Dihydrorhodamine (DHR) or Nitroblue tetrazolium reduction (NBT) assays. The median age at diagnosis was 1.75 years (0.1-14.7).
Ten of the 30 patients (33%) presented inflammatory manifestations during follow-up. The median age of diagnosis of CGD of patients with IM was 6.1 years (0.2-12.4) and the age of onset of IM ranged from 0.2 to 12 years (median 7.1y). In 4 out of the 10 patients, IM occurred before the diagnosis of CGD, with a median time between IM and the diagnosis of 5.85 years (2.1-7.4).
The IM diagnosed were inflammatory bowel disease-like (n = 5, with perianal fistula in 2/5), mouth ulcers (n = 1), discoid lupus (n = 1), autoimmune dermatitis (n = 1) and eczema (n = 1), chronic lung disease (n = 2) and granulomas (pulmonary n = 2; ocular n = 1; bladder n = 1; oropharynx n = 1). Three patients presented more than one site of inflammatory disease. All patients were treated with systemic or topical immunosuppressive or immunomodulatory therapy, most of them corticosteroids. Five patients underwent hematopoietic stem cell transplantation (HSCT), median age at HSCT was 13 years (4 - 17), and two died 1 month after HSCT.
Conclusions: Although infections are more frequent and have a major impact on patient morbidity and mortality, IM are increasingly prevalent in patients with CGD. Awareness regarding this possible comorbidity is of major importance, since earlier diagnosis and adequate treatment may be crucial for patients survival and quality of life.

(128) Submission ID#811299

Rheumatological Diseases in Patients with Primary Immunodeficiency Disorders in the USIDNET Registry

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Abstract/Case Report Text

Rationale: There is a gap in clinical knowledge regarding associations between specific PID and different rheumatological diseases. In this study, we are reporting the incidence of various rheumatological conditions reported in a large PID population using the USIDNET (United States Immunodeficiency Network) registry.
Methods: We used the retrospective USIDNet registry to conduct the analysis. We included all primary immunodeficiency patients with physician diagnosed rheumatological diseases.
Results: The total number of PID patients in our query was 5058. 278 (5.49%) patients had a diagnosis of rheumatological disease. This cohort included 172 (61.8%) female and 106 (38.2%) male patients. Rheumatologic complications were highest in the interferonopathies (66.6%), complement deficiencies (14.2%) and Autoimmune Lymphoproliferative Syndrome (ALPS) (13.7%). Additionally, disease patterns were noted to be different in each PID. Dermatomyositis was found to be the most common rheumatologic condition in patients with X-linked Agammaglobulinemia (XLA) with a rate of 1.65%, which was remarkably higher than the reported prevalence in the United States (0.005%). ALPS patients had a higher (6.85%) numbers of Sjogren Syndrome diagnoses as compared to the general population (0.01-0.09%). Systemic lupus erythematosus was increased in patients with Mucocutaneous Candidiasis (7.41%) as compared to the general population (0.001%) and other PIDs. Rheumatoid arthritis (RA) was reported in patients with Specific Antibody Deficiency (3.66%), Common Variable Immunodeficiency (CVID) (2.93%) and ALPS (2.74%). Wiskott-Aldrich Syndrome patients had the highest numbers of cases diagnosed with vasculitis (6.50%). 0.29% of patients with Severe Combined Immunodeficiency (SCID) had reported rheumatologic disease. Juvenile rheumatoid arthritis (JIA) and systemic sclerosis were reported in 0.19% of patients with DiGeorge Syndrome.
Conclusions: This study reports that higher numbers of rheumatologic diseases are diagnosed in PIDs compared to the general population. The incidence of different rheumatological disease was variable based on the PID diagnosis. Early diagnosis of these diseases is crucial, given the high risk of irreversible complications. Limitations of our study include possible selection bias as majority of cases were enrolled from tertiary care centers.

(129) Submission ID#811309

Hematopoietic Stem Cell Transplantation for Diseases of Immune Dysregulation – The Journey of the Cure for Hitherto Unrecognized Conditions

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Abstract/Case Report Text

Background Disorders of immune dysregulation are associated with autoimmune features. This feature could potentially have an impact on the outcome post hematopoietic stem cell transplantation (HSCT). HSCT, although curative, can be challenging with the underlying immune dysregulation resulting in significant morbidity and mortality. We present the journey through HSCT for these children and the factors affecting the outcome.

Patients and methods
We analysed the data on children up to the age of 18 years diagnosed to have a disorder of immune dysregulation through gene mutation analysis and who underwent HSCT at our centre from 2015 to 2019.
Results 1. XIAP mutation
A 10-year-old boy underwent a haploidentical HSCT from his father using fludarabine, treosulfan, and 2 Gray radiotherapy with post-transplant cyclophosphamide. After initial complete chimerism and cytomegalovirus reactivation responsive to valganciclovir, he developed progressive diarrhoea almost 15 months post-HSCT. A rectal biopsy confirmed CMV reactivation and features of inflammation. He has since been treated for the same and is on follow up for inflammatory bowel disease. His chimerism had dropped to 67% and has remained stable.
The second child is a 1-year-old girl who underwent TCR alpha/beta depleted haplo SCT and is 12 months post-HSCT, with no features of GvHD or infections, and is doing well with complete chimerism.
2. IL10R deficiency
Three boys aged eight months, one year, and two years of age, diagnosed to have IL10R deficiency underwent HSCT. All three children needed nasogastric tube feeding, parenteral nutrition, and vigilant monitoring for electrolyte disturbances. In the first two children, we had performed TCR alpha/beta depleted PBSC transplants from their haplo matched fathers. The 1-year-old engrafted by D+17 and is doing well two years post HSCT with complete chimerism, no GvHD, and infections. His autoimmunity, including recurrent skin scarring, has resolved entirely. The 8-month-old, however, had primary graft failure and succumbed to his illness.
The 2-year-old boy underwent matched unrelated donor HSCT and engrafted by D+14 with completed chimerism documented on three occasions. He, however, had secondary graft failure around D+60, and he succumbed to the illness.
3. LRBA deficiency
An 18-month-old girl with LRBA deficiency had presented at four months of age with excessive sweating, hepatosplenomegaly, and recurrent chest infections. She was started on monthly intravenous immunoglobulin replacement and Abatacept. She received myeloablative conditioning with thiotepa, treosulfan and fludarabine and underwent a matched sibling donor HSCT. She engrafted by D+17 and has been well ten months post HSCT with complete chimerism, no GvHD, and infections.
Conclusion Disorders of immune dysregulation are a heterogeneous group with a varied spectrum of immune dysfunction. Myeloablative conditioning is essential, and there is a high risk of cytokine release syndrome and the need for supportive care. The autoimmune features need to be followed for progression in organs other than the hematopoietic system and may require interventions. As long-term data evolves, more precise definitions for patient and donor selection will enable improving outcomes.

(130) Submission ID#811321

"One size does not fit all" - Hematopoietic Stem Cell Transplantation for Genotypic Variants of Severe Combined Immune Deficiencies

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Abstract/Case Report Text
Background We present a series of rare variants of SCID, their spectrum of clinical presentations, and challenges during hematopoietic stem cell transplantation (HSCT).

Patients and Methods
We performed a retrospective observational analysis of case records of children up to 18 years of age, diagnosed to have variants of SCID, and underwent HSCT at our centre from 2002 to 2019.
Results
1. ZAP 70 deficiency
A 6-month-old girl presented with oral thrush and submandibular cellulitis from one week of life with failure to thrive. She underwent a TCR alpha/beta depleted haploidentical HSCT. Conditioning included treosulfan/thiotepa/fludarabine/anti-thymocyte globulin. She engrafted by D+15; now three years post-HSCT with complete donor chimerism without GvHD or infections.
2. ORAI-1 mutation
A 15-month-old girl presented with failure to thrive, generalized hypotonia, oral thrush, and recurrent respiratory infections. She underwent haplo-SCT with post-transplant cyclophosphamide with PBSC from her haplo-matched father. Conditioning included fludarabine/treosulfan. She had cytokine release syndrome grade 4, which responded to tocilizumab. She had hypertension throughout the peri-engraftment period and had an episode of PRES with seizures. Her symptoms abated with neutrophil engraftment by D+17. The post-transplant period was complicated by grade 2 skin GvHD and cytomegalovirus reactivation. She has remained disease-free with complete chimerism three years post-HSCT. Her hypotonia is steadily improving with physiotherapy.
3. Cernunnos-XLF deficiency
A 27-year-old male presented with recurrent infections from 14 years of age, aplastic anemia diagnosed at 20 years of age, subsequent transformation to acute myeloid leukemia at 27 years of age. He had developed multiple fusarium abscesses during the neutropenic period post-chemotherapy for AML. He was referred for a matched sibling sister HSCT when in remission. Conditioning included fludarabine/treosulfan. He engrafted by D+15 with complete chimerism. He developed progressively worsening skin, gut, and liver toxicity secondary to chemotherapy and succumbed to the illness two months post-HSCT.
4. IKZF mutation
An 18-month-old girl presented with failure to thrive, massive splenomegaly, persistent pneumonia, anemia, and thrombocytopenia. She underwent a matched sibling donor PBSC transplant after myeloablative conditioning with thiotepa/treosulfan/fludarabine. She engrafted by D+20, following which all her symptoms abated. She had secondary graft failure two months post-HSCT and succumbed to her illness.
5. MHC Class II deficiency (bare lymphocyte syndrome)
Three children, aged 18 months, two years, and four years underwent matched sibling donor HSCT. Myeloablative conditioning with thiotepa/treosulfan/fludarabine resulted in engraftment. The first child died of invasive intestinal aspergillosis 30 days post-HSCT. The other two children are well 11 months post-HSCT with complete chimerism without GvHD or infections. The two-year-old girl received one cycle of pre-transplant immunosuppression with fludarabine/dexamethasone to prevent graft rejection pre-HSCT as she was referred for a second transplant.
Conclusion
Children with SCID have traditionally been transplanted using reduced intensity (RIC) conditioning with immunomodulation. SCID variants require myeloablative conditioning with a vigilant follow up for the detection of graft rejection. Radiation sensitive SCID associated with DNA breakage repair defects require RIC and close monitoring for GvHD. Advances in HSCT, including supportive care and haplo-SCT, have provided a ray of hope for these hitherto rare conditions.

(131) Submission ID#811347

Patient with Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) Syndrome Is Safely Treated with Tofacitinib

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Abstract/Case Report Text

Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) (OMIM #304790) is a monogenic autoimmune disorder that occurs due to loss of function variation in FOXP3 causing dysfunctional T regulatory cells. Although immunosuppression is a mainstay of treatment for autoimmunity, IPEX treatment is frequently limited by insufficient response to therapy or side effects of immune suppression. We present a 28 year old male with IPEX whose prior immunosuppressive treatment was complicated by inefficacy and medication side effects, requiring a new approach to treat his colitis and erosive dermatitis.

He initially presented with infantile diabetes and subsequently developed dermatitis, squamous cell carcinomas, alopecia totalis, and colitis. His clinical diagnosis of IPEX was confirmed by FOXP3 sequencing, demonstrating known pathogenic variant c.1150G>A (p.Ala384Thr). This variant has been described in IPEX affected individuals in multiple publications (Ref 1). His variant affects at the FRKhead domain of FOXP3 and has been associated with others with severe psoriasiform dermatisis and alopecia universalis (Ref 2).

His prior immunosuppressive therapies included at different times combinations of corticosteroids, tacrolimus, sirolimus, azathioprine, infliximab, adalimumab, rituximab, dupilumab, and oral mesalamine. The relative efficacy of these agents based on experiences in a cohort of IPEX patients was reviewed in 2018 (Ref 3), with the exception of duplimab, which was not listed in that review. For our patient, management of his widespread autoimmunity has been limited by toxicity or lack of efficacy of medications. Notably, his dermatitis had no improvement with duplimab, consistent his low total IgE and lack of allergic manifestations. At age 27, after initiation of treatment with sirolimus, he had spontaneous colonic perforation requiring descending colectomy.

After stabilization of his colonic perforation, his multi-disciplinary team of allergy-immunology, gastroenterology, and dermatology initiated tofacitinib. Tofacitinib is small molecule inhibitor of Janus kinase (JAK) signaling pathways that mediate cytokine driven autoimmune activation. It is FDA approved to treat Rheumatoid Arthritis, Psoriatic Arthritis and Ulcerative Colitis. The decision to use this JAK inhibitor was due to its FDA approved use for ulcerative colitis, to target our patient's colitis and his other autoimmune manifestations, specifically his dermatitis. Its off label for primary immune dyregulatory disorders including CANDLE, STAT1-Gain of function and STAT3-Gain of function disorders has been published (Ref 4), but thus far its use to treat autoimmunity due to IPEX has not been published.

He experienced leukopenia while on 15 mg of tofacitinib, which resolved after lowering his dose. Currently, he has had improvement in his colitis and dermatitis, and partial improvement in alopecia. He has been on tofacitinib 10mg daily for 11 months, with only prednisone 10mg daily as additional immune suppression.

As the number and types of selective immune modulators increases, there is continued need to share the experiences of treating physicians of which therapies have been successfully able to decrease disease manifestations with tolerable side effect profiles. We present a 28 year old male with IPEX Syndrome with severe dermatitis and colitis complicated by colonic perforation despite standard immunosuppressive therapy, who is safely and effectively being treated with tofacitinib.

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Lupus and Lupus-like Autoimmunity in Loss of Function STAT3

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Abstract/Case Report Text

INTRODUCTION Autoimmunity and auto-inflammation are common complications of primary immune deficiencies, manifesting frequently as inflammatory bowel disease, hepatitis and cytopenias. Dominant negative mutation in STAT3 (LOF STAT3; AD-HIES) is not frequently associated with autoimmunity, likely due to impaired IL-6 and IL-17 pathways. However, in our relatively large cohort of LOF STAT3 patients, we have noted an increased incidence of systemic lupus erythematosus (SLE) diagnoses and SLE- like symptoms. Herein, we characterized the clinical and laboratory features of the patients in our cohort with SLE and SLE-like disease, with the aim to better understand the pathogenesis by evaluating IFN stimulated genes and neutrophil net formation.

METHODS A retrospective chart review was performed of patients with LOF STAT3 to identify those with SLE and SLE-like presentations, and included clinical features, laboratories including inflammatory markers, auto-antibodies, and complement levels. RT-PCR was performed for interferon stimulated genes (ISGs) from neutrophils and PBMCs of LOF STAT3 patients with and without SLE, and healthy controls. Neutrophil NET formation was assessed for LOF STAT3 patients with and without SLE, and healthy controls.

RESULTS Out of a cohort of 158 patients, five patients (ages 12-39) were identified who carried the diagnosis of SLE, and 4 with SLE-like disease (ages 15-34). For those with SLE, age of presentation was 8-21 years, 4 of 5 were female. Clinical features included nephritis (4), alopecia (2), autoimmune cytopenias (2), arthritis (3), discoid rash (2), and Raynaud (1). All had positive auto-antibodies, and 4 of 5 had low C3 and/or C4. For those with SLE-like disease, age of presentation was 12-24, and 2 of 4 were female. Clinical features included alopecia (1), autoimmune cytopenias (1), Raynaud(1), and nephritis (2). All had positive autoantibodies, and 1 of 4 had low complements. LOF STAT3 patients with and without clinical features of SLE had increased expression of ISGs from both PBMCs and neutrophils. Increased spontaneous NET formation was observed for LOF STAT3 patients both with and without SLE symptoms.

DISCUSSION

Although autoimmunity is not a common finding in LOF STAT3, we have identified SLE or SLE-like disease in about 6% of our cohort, with a high incidence of kidney disease, including one patient who required kidney transplant. The interferon signature and NET formation were unexpectedly high in both the patients with and without the SLE features. Ongoing studies include whole

exome sequencing for possible second mutations or modifiers, the role of IgE in the kidney disease, and further autoantibody detection. The increased IFN signature raises the question about JAK-STAT modulation for therapy.

(133) Submission ID#811400

Differential expression of p67phox/p40phox In Neutrophils from Patients and Carriers With p67phox (gene NCF2) Chronic Granulomatous Disease By Fluorescence-Activated Cell Sorting

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Abstract/Case Report Text

Chronic granulomatous disease (CGD), a rare immunodeficiency with decreased reactive oxygen species (ROS) production, increased susceptibility to infection, and increased mortality is caused by mutations in any one of 5 distinct phagocyte oxidase (phox) components of the NADPH oxidase, NOX2. In the past, identification of the specific protein defect was primarily determined by immunoblotting using specific antibodies to the phox proteins. Recently, however, we have shown using fluorescence-activated cell sorting (FACS) analysis of neutrophils in whole blood permeabilized and stained with specific anti-p47phox antibody that p47phox protein expression was absent in p47phox CGD patients and significantly reduced in p47phox CGD carriers [Kuhns et al. 2019. Blood Adv. 3(2):136-147]. These findings demonstrated that determination of phox protein expression by FACS analysis provide an alternative to immunoblotting and can aid in the identification of p47phox CGD patients and carriers. We now have extended these studies to patients and carriers with p67phox CGD. FACS analysis of p67phox expression in permeabilized neutrophils demonstrated that p67phox expression was absent in four patients with different mutations in NCF2 [two patients homozygous for c. E12(+1) G>A, one patient homozygous for c.287_289 del AAG, p.Glu96 del; and one patient compound heterozygous for the mutations, E3(+1) G>A and c.55_63 del AAGAAGGAC]. Moreover, the expression of p67phox in nine p67phox CGD carriers was significantly reduced >50% compared to expression in neutrophils from healthy volunteers. Another cytosolic phox protein, p40phox, has been shown to associate with p67phox in a 1:1 molar ratio [Tsunawaki et al. 1994. Biochem Biophys Res Comm. 199(3): 1378-1387]. The expression of p40phox was reduced in both carriers and patients with mutations in NCF2. Despite reduced expression of p67phox and p40phox, neutrophils isolated from carriers of p67phox CGD exhibited normal dihydrorhodamine (DHR) oxidation after stimulation with phorbol ester and fell within the normal range for ROS production (measured by luminol-enhanced chemiluminescence) after stimulation with either fMLF, opsonized zymosan, or phorbol ester with one notable exception. Included in this cohort of p67phox carriers was a p47phox CGD patient (homozygous for a GT deletion at the start of exon 2 in NCF1) who also carried a heterozygous damaging mutation in NCF2 [c.1256 A>T; p. Asn419Ile]. Normal ROS production in the presence of reduced p67phox and p40phox expression suggest that these proteins are not rate-limiting components for maximum NOX2 activity in neutrophils. Finally, determination of the expression of specific phox components by FACS analysis of permeabilized neutrophils from whole blood provides a rapid and alternative approach to immunoblotting to determine the specific protein defect in CGD, and, importantly, one that could be easily established in most clinical labs.

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Impaired CXCR2-specific Neutrophil Chemotaxis and Elevated STAT1/2 Expression in Neutrophils and Monocytes from Hyper-IgE Syndrome Patients

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Abstract/Case Report Text

The original clinical observation that defined patients with Hyper-IgE syndrome (HIES) was the presentation of cold abscesses ("Job's syndrome"), which indicated a deficient inflammatory response. Mutations in the STAT3 gene have now been identified in most classic autosomal dominant HIES patients, but we do not fully understand how these mutations cause the clinical presentation. Since the discovery of STAT3 mutations, research on HIES focused largely on the adaptive arm of the immune system and suggested that the innate immune defects could be secondary. For example, the discovery that there is a Th17 cell and IL-17 cytokine deficiency in HIES provided a possible explanation to the neutrophil chemotaxis defects in HIES, as IL-17 is one of the chemokines critical for neutrophil recruitment in vivo. The goal of this study was to investigate myeloid cells from HIES patients.

First we used C5a, fMLP, IL-8, CXCL1, and CXCL2 to study neutrophil chemotaxis in vitro. Responses to C5a, fMLP, and IL-8 were equally robust in HIES compared to healthy controls, demonstrating that neutrophils from patients are capable of efficient directed migration in vitro. Neutrophils from all HIES patients responded to CXCL1 and CXCL2 significantly below that of the healthy controls. CXCL1 and CXCL2 are CXCR2-specific chemokines. These results indicated a neutrophil intrinsic CXCR2-specific defect. We also found that patient-derived cells express comparable levels of CXCR2 on the cell surface, suggesting a CXCR2 chemokine receptor signaling defect.

After identifying a neutrophil defect in HIES, we wanted to get a broader view of myeloid cells in HIES in addition to identifying the CXCR2-specific defect. STAT3 is a transcriptional regulator, therefore we performed transcriptional profiling of HIES and healthy control-derived neutrophils and monocytes. As it was shown before, the expression of STAT3 was not different between patients and controls, since HIES is usually caused by the decrease in STAT3 activity not by decrease in expression. We found, however, an increase in STAT1 and STAT2 expression as well as significant changes in the expression of genes regulated by interferons. Increased expressions of STAT1/2 in both neutrophils and monocytes likely provide and explanation for the increase in interferon regulated genes. Multiple genes were identified as potential regulators of CXCR2 signaling.

The balance between the STAT3 and STAT1 signaling has long known to be a regulator of immune cell activation, especially in T cells, but less studied in myeloid cells. STAT3 and STAT1/2 signaling pathways cross-regulate each other in healthy cells. We propose that in HIES the

decreased STAT3 signaling leads to not only changes in expression of effector (e.g. inflammatory) genes, but also decreases expression of genes in the regulatory (negative) feed-back loop, which are required for decreasing STAT1/2 activity. Therefore, the immune cell defects caused by decreased STAT3 activity are compounded by the increase in STAT1/2 activity. Increase in STAT1/2 signaling can cause pathologies in the absence of STAT3 defects, as well as further decrease STAT3 signaling, thus contributing to HIES. Interfering with STAT1/2 signaling in HIES may represent a therapeutic opportunity.

(136) Submission ID#811689

Comparison of Mortality of Matched Newborn Infants with Low TRECs Levels of Unidentified Etiology

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Abstract/Case Report Text
Rationale: T-cell receptor excision circles (TRECs) testing on newborn screening (NBS) has been vital for identifying patients with severe combined immunodeficiency (SCID). We aimed to determine whether one or more abnormal TRECs result on a NBS might predict higher mortality rates despite the absence of an identifiable underlying etiology.
Methods: Newborns with a positive TRECs NBS result without the diagnosis of SCID or 22q11.2 deletion syndrome born from October 2011 to December 2014 were included (n=467). Newborns were divided into three groups: group 1 infants had a subsequent normal repeat screen (n=375); group 2 infants did not undergo repeat screening as the majority expired before a repeat screen could be conducted (n=36); group 3 infants had a normal initial screen but subsequent abnormal screen (n=56). Cases were matched 3:1 to controls on gestational age, birth weight, NICU status, race, birth quarter, and birth year. NBS records were linked to birth and death certificate records. Demographic characteristics were compared and mortality rates were calculated between the groups.
Results: The mortality rate of group 1 was 2.4%, group 2 was 91.7% and group 3 was 46.4%. When compared with matched controls, there was no difference in the mortality rate of group 1 when compared to the control group. There was a significant difference in the mortality rate between cases and controls in both group 2 ($p < 0.001$, 95% CI 0.711, 0.950) and group 3 ($p < 0.001$, 95% CI 0.256, 0.551). The APGAR scores in group 1 infants were comparable to their matched controls. Infants in group 2 ($p = 0.01$) and group 3 ($p = 0.003$) had significantly lower APGAR scores than the controls. The majority of the infants in all three groups were less than 37 weeks gestation, however, group 2 had a higher percentage of infants born very premature (less than 32 weeks). There was no significant difference in maternal age, maternal education, prenatal care status, cigarette use, or maternal steroid use between the cases and controls in all three groups.
Conclusions: Infants with an initial abnormal screen who had a subsequent normal repeat screen did not have an increased rate of mortality compared to their matched controls (group 1). However, group 2 infants (with unresolved repeat screen) and group 3 infants (with a first abnormal value on a repeat screen) did have increased mortality rates when compared to their controls. Overall, an abnormal TRECs level on NBS without a confirmed negative repeat screen, was associated with higher mortality in our study population. Further studies will be needed to determine if the TRECs assay can serve as a predictor for mortality in newborns with an abnormal screen.

(137) Submission ID#811713

Evaluation of immunoglobulin (Ig) G4 Deficiency in an Outpatient Clinic in Brasilia, Brazil

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Abstract/Case Report Text
INTRODUCTION: Primary immunodeficiency refers to a heterogeneous group of diseases characterized by altered function or

composition of the immune system, and are grouped into adaptive or innate system defect. Immunoglobulin G subclass immunodeficiencies (IgGSCS) are classified as a B-cell-related adaptive system disorder and are therefore associated with recurrent sinopulmonary infections with encapsulated bacteria, presenting with pneumonia, recurrent bronchitis, rhinosinusitis, and herpes zoster. Its primary mechanisms are still unclear, although the cause for this deficiency might be related to gene deletions, transcription errors, or be an effect of allotype. IgG4 immunodeficiency reaffirms its association with the patient's clinical condition and is often associated with IgG2 deficiency.
OBJECTIVE: To evaluate the prevalence of IgG4 immunodeficiency in Ferraroni's Clinic, classify it by gender, age, IgG4 dosage and other subclasses, correlate it with IgG2 immunodeficiency and the clinical presentations presented by the patients under analysis. METHOD Records of 24 patients with IgG4 immunodeficiency whose clinical pictures were followed throughout 15 years were evaluated, patients aged from 4 to 85 years. All tests were done at the same laboratory and all patients have consented to be part of this study, which has been approved by the ethics committee. RESULTS Twenty-four patients with IgG4 deficiency, 79,16% (n=19) were women and 20,83% (n=5) were male, with average of 47 and 28 years, respectively. The average of IgG4 was 7.76 mg/dL, and that of IgG2 was 299 mg/dL. Of the patients evaluated, 62.5% had upper airway infections (sinusitis, rhinitis, otitis and tonsillitis), 25% herpes simplex, 33.3% asthma. Less prevalent cases were reported as 4.1% of patients had bronchiectasis, 12.5% candidiasis and 4.1% herpes zoster. 41.67% presented the association of IgG4 and IgG2 deficiency.
DISCUSSION: The role of specific IgG4 deficiency in the infectious setting is still unknown, but it usually occurs in association with other isotypic deficiencies and sinopulmonary infections. Furthermore, the IgG4 subclass is relevant on the study of environmental antigens - suggesting its involvement with allergic disorders - and has been described in association with other diseases, such as chronic mucocutaneous candidiasis, ataxia-telangiectasia and allergic colitis. IgG2 deficiency is related to increased susceptibility to bacterial infections. Studies show a correlation between IgG2 and IgG4 immunodeficiency that generally imply clinical features characterized by recurrent infections by encapsulated bacteria. The data obtained through the analysis of patients' charts corroborated this information, since it was evident that most of the patients had really similar clinical conditions.
CONCLUSION: IgG4 deficiency has a direct correlation with higher prevalence of upper airway infections, such as rhinitis, sinusitis and pneumonia, and with an increased incidence of allergic disorders, here presented by our cohort.

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T-Helper 17 Cells as a Diagnostic Indicator for Hyper-IgE Syndrome

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Abstract/Case Report Text
INTRODUCTION Hyper-IgE syndrome (HIES) caused by STAT3 loss-of-function (LOF) variants is characterized by eczema, skin abscesses, fungal infections, life-threatening pulmonary disease, and significantly elevated IgE levels. Small cohort studies demonstrated a decreased percentage of CD4+ T cells expressing IL-17 (Th17) in HIES; however, decreases in the percentage of Th17 cells were also seen in atopic disease. Interestingly, these studies also observed a lower percentage of CD4+ T helper cells expressing IFNg (Th1). Additionally, research suggests that HIES may cause impaired CD8+ T cell function. We hypothesized that a low percentage of both Th17 and Th1 cells would be predictive of HIES and would differentiate HIES from atopic disorders. To evaluate this hypothesis, we examined the percentage of Th17, Th1, and IFNg+CD8+ T cells, laboratory parameters, and genetic diagnoses from a large cohort of patients to determine which parameters distinguish patients with STAT3 loss-of-function variants.
METHODS: We conducted a retrospective, multi-institutional chart review of over 200 patients who received a Th17 assay at the Medical College of Wisconsin Clinical Immunology Research Laboratory. The Th17 assay is performed by activating PBMCs with PMA/ionomycin/brefeldin A and staining for CD4, CD8, IFNg and IL-17A. The following parameters were included in the chart review: the percentage of Th17, Th1, and CD8+IFNg+ cells, immunoglobulin levels, atopy scores, infectious history, and genetic diagnoses.
RESULTS: Using logistic regression, we demonstrated that the percentage of Th17, CD8+IFNg+, and Th1 cells were positively correlated with age, and percentage of CD8+IFNg+ cells was higher in females than males. We found that the percentage of Th17 and Th1 cells were decreased in both atopic disease and HIES, with HIES having the lowest values. Interestingly, one subject with a STAT3 gain-of-function (GOF) variant had an elevated percentage of Th17 and Th1. In addition, we determined that IgE levels were inversely correlated with the percentage of Th17, CD8+IFNg+, and Th1 cells, while IgA and IgM were positively correlated with the percentage of Th17 cells. Several different monogenic defects characterized by increased fungal infections exhibited a low percentage of Th17 including Tatton-Brown-Rahman syndrome and Cornelia de Lang syndrome.
CONCLUSIONS:
We confirmed that the percentage of Th17 cells is low in both HIES and atopy in a large cohort of subjects, and that the percentage of Th1 cells may be helpful in distinguishing HIES from atopic disease. However, since the percentage of Th17, CD8+IFNg+, and Th1 cells correlate with age, caution should be used when testing young children. The inverse correlation between IgE levels with Th1 and Th17 responses suggests that similar pathway(s) may drive both HIES and atopy. Additionally, the decreased Th1 responses in STAT3 LOF and increased Th1 responses in STAT3 GOF HIES raise questions about the role of STAT3 in regulating IFNg levels. We also identified patients with different genetic disorders with fungal infections in which the Th17 percentages were low, suggesting that the Th17 test may be useful in evaluating individuals with unusual fungal infections.

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Atypical Autoimmune Neutropenia: Data from The Italian Neutropenia Registry

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Abstract/Case Report Text

Background:Primary and Secondary Autoimmuune Neutropenia (pAN/sAN) are well described entities. Several Autoimmune Neutropenias do not fit the criteria of either pAN or sAN showing peculiar characteristics mainly for older age at onset and/or for duration of the disease; moreover they are not associated , at least at the beginning, with autoimmune markers/diseases Aim of the study: to describe a cohort of subjects affected with autoimmune neutropenia, defined as “atypical” (aAN), registered in the Italian Neutropenia Registry (INR) and to compare these data with those from subjects diagnosed with pAN still in the INR.

Patient and methods: Subjects with neutropenia and positivity of indirect antibodies against neutrophils (registered in the INR from 2013 to 2019) lasting for more than 3 years, or diagnosed after 5 years of age (up to 18 y), without any associated autoimmune, signs/markers were considered eligible for the present study.

Results: Data from 248 patients were collected: 79/128 subjects (32%) were defined as aAN and 169/248 (68%) as pAN. Among 79 aAN affected patients 61%, were “long lasting” aAN, while 39% were defined as “late onset” aAN .The degree of neutropenia in aAN group was mild in 20 %, moderate 44 % and severe in 36 % of the subjects . Leukopenia at onset was a common hall mark seen in 46% of aAN patients (median values 3700/mm³ ; range 2750-5140/mm³) especially in the “late onset” aAN if compared with pAN and “ long lasting” one (p=0.003). As for clinical features, almost half of the aAN cohort suffered from recurrent or “significative infections”, while severe episodes (namely sepsis, meningitis , osteomyelitis , pneumonia, deep abscess or flemmon) were shown in 28% being more frequent, but non significantly higher than those reported in the pAN group (6%)(P=ns) Interestingly, recurrent apthae were significantly more seen in the “late onset” aAN group if compared with the “long lasting” aAn (P=0.006). During follow up, markers and/or symptoms of autoimmunity appeared in 31% of the aAN cohort, being another element of peculiarity in respect to pAN ($p < 0.0001$).

As for immunological pattern in aAN, immunoglobulin values were lower than the references for age in 12%,while were above them in 17% of the cohort . Lymphocytes subsets evaluation showed decreased value of CD3+CD19+ cells in 40% of cases, followed by depletion of CD3-CD16+CD56+ subtype in 33%, CD3+CD4+ in 24 % of cases and CD3+ CD8+ in 17% . Preliminary study on B memory and T-reg cells values, showed a quantitative deficiency respectively of in 59% and 27% of the studied subjects.

Mutation analysis performed by NGS in 20% of the subjects identified pathogenic variants of : TACI (2), TINF 2 (1) and LRBA(1) .Comparison between pAN and aAN is detailed in Table 1.

Conclusions Atypical neutropenia in childhood is a disorder which show many difference with pAN; indeed appears an epiphenomenon of a complex immunological disturbances rather than a disease itself. Occasionally mutations of genes of immunodeficiency/disimmunity can be demonstrated

Table 1 Differences between Primary AN and Atypical AN

	pAN	aAN	p
Sex(F)	59/169	38/79	0.01
Duration of Neutropenia(IQR)	1 (0.5-1.6)	3.9 (2.6-5.6)	0.0000
Severe or recurrent infections	80/163 (49%)	32/76 (42%)	0.31
Appearance of Autoimmunity disease or markers during follow up	3/169 (1.8%)	24/77 (31%)	0.01
Median Absolute Neutrophil Count (ANC) at onset (IQR)	430 (230-685)	600 (400-860)	0.001
Median ANC in follow up (IQR)	400 (230-690)	600 (400-870,5)	0.0001
Leukopenia at onset	14/157 (9%)	33/72 (46%)	<0.0001
Median Lymphocytes value (IQR)	4735 (3510-5840)	2150 (1561-3010)	0.0000
Monocytosis at onset	23/145 (16%)	15/64 (23 %)	0.19
Resolution of neutropenia	135/169 (80%)	32/79 (40,.%)	<0.0001
Bone Marrow examination	52/167 (31,1%)	44/70 (63%)	<0.0001
G-CSF Treatment	9/166 (5%)	10/65 (15%)	0.01
CD3+ value below the 5°percentile for age	9/86 (10,5%)	13/63 (21%)	0.08
CD3+CD4+ value below the 5°percentile for age	10/88 (11%)	15/63 (24%)	0.04
CD3+CD8+ value below the 5°percentile for age	13/88 (15%)	11/65 (17%)	0.72
CD3+CD19+ value below the 5°percentile for age	9/80 (11%)	25/62 (40 %)	<0.0001
CD3-CD56+CD16+ value below the 5°percentile for age	13/79 (164%)	19/57 (33%)	0.02

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Increased Rate of Secondary Immune Deficiency In Rheumatoid Arthritis (RA) Patients Receiving DMARD Therapies

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Abstract/Case Report Text

Background: Medications treating RA typically include systemic corticosteroids used to treat inflammation flares, and disease modifying therapies (DMARDs). Traditional DMARDs include methotrexate, leflunomide, hydroxychloroquine, and sulfasalazine. Recently, biologic/immune-response modifiers have come to the forefront for overall therapeutic benefit, however, an unfortunate side-effect may be the risk of increased immunosuppression. This study seeks to determine the occurrence rates of immune deficiencies among patients initiating RA therapies.
Methods: Using the Pharmetrics Plus commercial claims database from 2012-16, RA patients (ICD-9 714 and -10 codes: M05, M06) over the age of 18 were indexed on their first use of a new biologic therapy. All patients were required to have enrollment six-month pre and one-year post index. Cohorts of patients were grouped by medication: methotrexate, adalimumab, etanercept, and rituximab. RA patients receiving adalimumab, etanercept, and rituximab were allowed concomitant use of methotrexate, but could not use any other biologic medications in the post period. A minimal adherence of 40% was required of all biologic treated RA patients. An additional cohort of RA patients untreated with biologic therapies was indexed on their first RA diagnosis within the time window and used as a control. RA patients with comorbid conditions who would also require biologic treatment were excluded including Crohn's disease and ulcerative colitis. Between group comparisons were made with the no treatment group as the referent. To account for differences in age, gender, and Elixhauser comorbidity conditions patients in each cohort were matched 1:1 to the rituximab group.
Results: 52,013 RA patients met inclusion criteria: 6,608 in the methotrexate group, 2,826 receiving etanercept, 2,808 receiving adalimumab, and 467 receiving rituximab. A total of 12,709 in the treated groups and 39,304 in the no biologic treatment group. Demographic information including age and gender were significantly different but numerically similar between the groups, with rituximab group having the highest proportion of female patients but limited dispersion with the lowest proportion being in the etanercept group. Healthcare utilization metrics highlighted a significantly higher average number of office visits (21.18, SD: 13.48 vs no treatment 15.61, SD: 13.29, $p < 0.01$) and a higher proportion of rituximab patients being hospitalized (14.35% vs no treatment 10.37%, $p < 0.01$). The diagnosis of immune deficiency was highest among the rituximab group with 7.92% followed by methotrexate 2.91%, adalimumab 2.88%, etanercept 2.80%, and no treatment 2.80%. After matching, similar rates were seen for healthcare utilization to the pre-match results. The post-match odds of being diagnosed with immune deficiency were significantly greater for the rituximab group (OR 3.76, CI: 1.61-8.85) than the no treatment group.
Conclusions: The purpose of DMARDs is to modulate the immune system and decrease autoimmunity in RA. However, this treatment may lead to significant immunosuppression. This study suggests that treatment with certain biologic/immune-response modifier therapies may be associated with higher rates of healthcare utilization. In particular, the increased post-treatment diagnostic coding of immune deficiency demonstrates the heightened awareness among healthcare providers of the chronic immunosuppressive potential of rituximab. Evaluation of potential secondary immunodeficiency pre- and post-DMARD use should be incorporated into routine practice.

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Autoimmune Lymphoproliferative Syndrome (Alps) Disease and Alps Phenotype: Are Distinct Entities?

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Abstract/Case Report Text

Introduction: Autoimmune lymphoproliferative syndrome (ALPS) is a rare inherited disorder of lymphocyte homeostasis due to a FAS-mediated apoptosis and characterized by non-infectious and non-malignant lymphoproliferation, autoimmunity, and secondary malignancies (National Institute of Health criteria). In spite of recent progress, one third of ALPS patients still remain gene orphan and they have been previously categorized as ALPS-U. In some cases, patients fitting ALPS diagnostic criteria have been shown to carry mutation on genes involved in other immune-dysregulation syndromes.

Aims: The aim of this study is to compare the clinical and immunological features, and the outcome of a cohort of ALPS patients with mutations on the typical causing genes (FAS, FASL, FADD and CASP 10)- here defined as ALPS-G - vs the ones without a molecular diagnosis or carrying mutations on other genes (both defined as ALPS-U).

Patients and methods The demographic, clinical, biochemical, genetic informations and details about treatment are derived from the ALPS Italian Network. Search of mutations was performed with Sanger PCR and/or Next Generation sequencing techniques (extended to immunodeficiency genes panel).

Results: 68 ALPS patients were registered in our data base; the genetic analysis was performed in 42 subjects (62%): 14/42 pts (33%) were ALPS-G and the remaining 28 (66%) ALPS-U. Six-teen out of 28 (57%) ALPS-U patients resulted to carry mutations on other genes (LRBA, STAT3+CECR, CTLA4, BAFFr, TACI, NMLRC4, IKBKG, Gaucher), and the remaining 12 (43%) were negative.

The ALPS-U subjects showed a more complex phenotype compared to the ALPS-G group, which was characterized by multi-organ involvement (p=0.003) and positivity of autoimmune markers (p=0.002). (Table 1). Cytopenia affecting one or more haematopoietic lineages was present in both groups (69% and 82%) with no significant difference, apart from lymphocytopenia that was more frequent in ALPS-U group (p=0.03) (Table 1). As for lymphocyte subets and immunoglobulin dosage no differences were shown within the two groups. Vitamin B12 and IL-10 were more frequently raised in ALPS-G group (p=0.01, p=0.001) (table 1). Four out of 42 (9%) patients did not require any treatment. First-line treatment (steroid or intravenous immunoglobulins) controlled the disease only in 4/38 (10%) cases. The response rate to second line therapy -micofenolate mofetile (MMF) or rapamycin- was 100% and 40% in ALPS-G and ALSP-U group, respectively. Moreover, target therapies or drug combinations were more commonly applied in ALPS-U subjects (p=0.02) (Table 1).

Conclusions: Our study showed that ALPS-U subjects, despite the ALPS phenotype, represent distinct clinical entities and that genes associated with other immune-dysregulation syndromes are frequently represented in this group (16/28, 57%). The identification of such disorders is crucial for the management of second-line treatment and/or the administration of target therapies

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Mechanism(S) Of Prolonged Attenuation of Allergic Responses After Modulation of Idiotypic Regulatory Network

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Abstract/Case Report Text

Background: We have shown previously that allergic reactivity to ovalbumin (OVA) could be regulated in mice following perturbation of immune networks using combinations of an immune Ig along with anti-idiotypic Ig. We have explored features of this regulation including: its persistence after cessation of administration of combined Igs; the ability of heterologous Igs to produce immunoregulation; a role for Treg induction in regulation; and the ability to attenuate responses in mice pre-sensitized to an allergic stimulus.

Methods: BALB/c mice were sensitized to OVA. Mice also received 5 weekly injections of immune Ig or anti-idiotype Ig (at separate sites) from either homologous (mouse) or heterologous (human) sources. In the latter case pooled IVIG (given IM, hence hereafter IMIG) was used as a source of anti-idiotype Ig, and human anti-Tet as immune Ig. Injections of the Ig were given from the time of OVA sensitization (to attenuate development of immunity), or after pre-sensitization of mice (to attenuate existing allergic responses). All mice were assayed for development of OVA-specific serum IgE and IgG, as well as the production of OVA-induced IL-2, IL-4, IL-13, IL-31 and IL-33 in splenocytes cultured for 72hrs. In studies examining possible mechanism(s) responsible for inhibition of immunity mice received, in addition to the Ig treatments described, infusion of depleting anti-CD4, and/or anti-CD8 antibodies, or a mAb to TNFSFR25, known to expand Tregs implicated in regulation of Allo immunity.

Results: Combinations of both heterologous and homologous immune Igs and anti-idiotype Igs attenuated OVA allergic responses in both naïve and pre-sensitized mice. This attenuation persisted in mice greater than 14 weeks after cessation of treatment with the Igs used. Finally, depletion of either CD4 or CD8 cells ameliorated the suppressive effect seen, while the combination of anti-CD4 and anti-CD8 essentially abolished suppression. Suppression was further enhanced by anti-TNFSFR25 mAb.

Conclusions: We conclude that the combine Ig treatment protocols used produced a long-lasting suppression of allergic immunity, even in pre-

sensitized animals. The effects seem to depend upon induction and expansion of Tregs and represents a novel approach to treatment of allergic disease in humans and other animals.

(144) Submission ID#811811

T Cells Utilize Somatic Mutations and Epigenetic Silencing To Evade Nuclear-Retained WASp

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Abstract/Case Report Text
Background Wiskott-Aldrich Syndrome protein (WASp) is found in the cytoplasm of hematopoietic cells but can transit to T lymphocyte nuclei at distinct developmental timepoints. WASp deficiency is a rare, X-linked combined immunodeficiency disease. Affected patients display qualitative but not quantitative T cell defects.
We report two immune deficient subjects with nearly identical exon 11 frameshift mutations in WAS, the gene encoding WASp. One subject lacked circulating T cells, the other possessed several distinct CD8 T cell populations each expressing quantitatively different amounts of WASp.
Objective To determine how similar WAS mutations can cause SCID in one person and generate B and T cells with heterogenous WASp expression in another.
Methods To identify somatic WAS mutations, we deeply sequenced WAS exons, introns, promoters and 5' untranslated regions at 10,000 read depth in genomic DNA from various B and T cell populations of each subject and their unaffected relatives. We confirmed genomic variants were transcribed and translated by sequencing WAS transcripts and analyzing WASp in primary cell lysates, both fractionated and not. To model our subjects' diseases we transfected primary cells and cell lines with mutant WAS transcripts and then measured viability and nuclear localization via confocal microscopy.
Results Deep sequencing of genomic DNA revealed all of subject one's cells carried the same germline exon 11 frameshift WAS mutation. The mutation was incorporated into subject one's WAS transcripts and translated into a truncated form of WASp, which was relegated primarily to the cell nucleus.
Subject two possessed three distinct CD8 T cell subsets that each carried either the germline exon 11 frameshift WAS mutation or a variety of somatic mutations that circumvented frameshift WASp expression. Evasion strategies included exon 11 skipping, adoption of a cryptic exon 11 splice site and reversion to wild type amino acid sequence. Subject two incorporated somatic mutations into WAS transcripts which encoded either stable near full-length proteins or unstable non full-length ones.
Subject one's sister and subject two's mother, who both carried the germline exon 11 frameshift mutation, produced only wild type transcripts and proteins.
Conclusion We report two patients with WAS mutations encoding truncated WASp. If expressed, truncated WASp localized to the cell nucleus, and this was associated with T cell developmental arrest and Severe Combined Immune Deficiency. If, through a variety of epigenetic and somatic strategies, T cells could avoid expression of truncated WASp, they would survive but display phenotypical abnormalities and functional defects.

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Paediatric lymphomas: a possible warning sign of Primary Immunodeficiency Disorders (PIDDs)?

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Abstract/Case Report Text
Primary immunodeficiency disorders (PIDDs) and immune dysregulations are rare pathological conditions associated with a high risk of malignancy. Patients with PIDDs show a higher susceptibility to hematopoietic malignancies, in particular to Non-Hodgkin lymphomas (NHL) that, generally, account for approximately 6-7% of paediatric cancers and their incidence increases with age. Recently new gene defects responsible for PIDDs with lymphoproliferation as a key clinical sign have been identified.
Our goal is to investigate possible immune-mediated mechanisms underlying malignant lymphoproliferation in children who did not show other typical symptoms of PIDDs.
We retrospectively selected and reviewed the clinical history of nine patients with NHL (6 Burkitt lymphoma, 2 large B cell lymphoma and 1 lymphoblastic T cell lymphoma). Immunophenotyping and exome analysis of known PIDDs genes were performed after lymphoma remission.
Six out of nine patients showed a mild hypogammaglobulinemia at time of presentation, not noticed before. Moreover, one patient had history of recurrent respiratory infections, one of hematologic autoimmunity and two of nine were EBV-positive at diagnosis. Preliminary results show an aberrant B cell phenotype in four patients; exome analysis reveals a novel heterozygous genetic variation in IKZF1 gene in one patient with

Burkitt lymphoma and autoimmune cytopenia was identified. Concerning the remaining patients, further studies are ongoing.
A detailed review of clinical history of paediatric patients affected from NHL as well as an impaired immunophenotyping can be important indicators of immune-mediated disorder underlying lymphoproliferation and helpful signs of possible PIDDs that should promptly be investigated by genetic analysis. This will allow an appropriate diagnosis and disease management.

(146) Submission ID#811829

Dupilumab for Treatment of Caspase Activation And Recruitment Domain 11 Deficiency-Associated Atopic Dermatitis

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Abstract/Case Report Text
Introduction: Caspase activation and recruitment domain 11 (CARD11) encodes a scaffold protein that links antigen receptor activation to intracellular signaling. Dominant heterozygous loss of function (LOF) mutations in CARD11 cause a syndrome of severe atopic dermatitis, elevated IgE, and allergic disease. Atopic dermatitis can be difficult to control leading to substantial morbidity. Dupilumab is a humanized monoclonal antibody that blocks IL-4 and IL-13 signaling approved for treatment of refractory atopic dermatitis. We present a case of a 10-year-old female with CARD11 deficiency successfully treated with dupilumab.
Case: A 10-year-old Puerto Rican female with history of recurrent sinopulmonary infections with 5 episodes of pneumonia, moderate persistent asthma, food allergies, recurrent skin boils, and severe atopic dermatitis was referred for further management and evaluation for autosomal dominant hyper-IgE syndrome (AD-HIES). Her atopic dermatitis was refractory to conventional therapy with topical corticosteroids, twice-daily emollient use, and bleach baths; it was also refractory to immunosuppression with mycophenolate mofetil and cyclosporine. On exam the patient exhibited coarse facial features and a high palate. She had eczematous lesions on the face, trunk, and extremities (SCORAD 84). Laboratory evaluation showed: eosinophilia (1400 cells/uL), elevated IgE (>2000 kU/L), low IgM (20mg/dL), and elevated IgA (568 mg/dL). Lymphocyte subsets and mitogen response were normal but antigen-induced proliferation was abnormal. Autosomal dominant hyper IgE score was 42 indicating a high likelihood of AD-HIES. No mutations in STAT3 were identified and Th17 cell expression was elevated. Dedicator of cytokinesis 8 (DOCK8) deficiency was also considered but DOCK8 protein expression was normal. Further genetic testing revealed an 18 base pair deletion in CARD11 (c.518_535del) predicted to be pathogenic. The combination of the patient's phenotype and large deletion was consistent with CARD11 deficiency. Despite continued immunosuppression with cyclosporine and aggressive skin care, the patient's atopic dermatitis was still severe and poorly controlled. Off label (patient < 12 years) treatment with subcutaneous dupilumab 300mg every 2 weeks was initiated. At last follow-up, 4 months after dupilumab start, the patient had substantial improvement in dermatitis with clear skin on the face, trunk, and extremities (SCORAD 40). Cyclosporine was discontinued and topical medications were applied less frequently.
Discussion: Hypomorphic heterozygous dominant negative loss of function mutations in CARD11 have recently been associated with severe atopic dermatitis and allergic disease. Treatment of atopic dermatitis in CARD11 deficiency remains challenging, but dupilumab appears to be an effective alternative to refractory disease. Longer follow-up and a larger cohort of CARD11-LOF patients treated with dupilumab are necessary to understand the long-term efficacy and safety for use of dupilumab in these patients.

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Impaired Monocyte and Langerhans Cell Innate Immunity in Patients with Recurrent Respiratory Papillomatosis (RRP)

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Abstract/Case Report Text
Purpose: The micromilieu within premalignant respiratory papillomas supports persistent HPV6/11 infection and disease recurrence in recurrent respiratory papillomatosis (RRP). These patients show polarized (TH2-/Treg) adaptive immunity in papillomas and blood, enriched immature Langerhans cell (iLC) numbers, and overexpressed COX2/PGE2 in the upper airway. To better understand the adaptive and innate dysregulation in RRP, we studied blood-derived monocytes, iLCs, and tissue-derived iLCs from RRP patients and controls.
Experimental Design: Monocyte subpopulations were isolated, differentiated into iLCs, activated, and then assessed by flow cytometry. Monocytes were induced to differentiate into iLCs with/without added PGE2, and then activated by IL-36γ, PGE2, PGE2+IL36γ, or LPS. iLC CD83 expression was identified by flow cytometry. Monocyte-derived iLCs, papilloma, foreskin, and abdomen skin iLCs, were also analyzed by qPCR for select chemokine/cytokine mRNA expression after isolation, 24 hrs later in culture, and again after poly(I:C) or TNFα stimulation.
Results: The three monocyte sub-populations differed between patients and controls, and patients' monocytes generated fewer iLCs. Classical monocytes generated most, but not all iLCs. PGE2 levels were higher in RRP plasma, and added PGE2 reduced control, but not patients' monocyte-iLC differentiation. PGE2 had no effect on iLC maturation identified by CD83 expression. Papilloma-derived iLCs expressed low CCL-1, and high CCL-20 mRNA and were unresponsive to poly(I:C) or TNFα. Tissue-specific cytokine/chemokine responses between iLCS from papillomas, foreskin and abdominal skin differed. Only papilloma iLCs expressed IL-36γ after isolation, and they up-regulated CCL1 mRNA 24 hrs later without further stimulation.
Conclusions: Monocyte/iLC innate immunity is impaired in RRP, in part due to increased PGE2 exposure. The immunosuppressive papilloma micromilieu likely alters iLC responses that skew, HPV6/11-specific TH2/Treg adaptive immunity in RRP.

(148) Submission ID#811847

Different Phenotypic Expression of the Same Compound Heterozygous Familial Mediterranean Fever Mutation in Identical Twins

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Abstract/Case Report Text

Introduction: Familial Mediterranean fever is a hereditary auto inflammatory disorder that typically manifests with recurrent fevers, abdominal pain and in some patients there is an associated with amyloidosis leading to eventual renal failure. While there are several common mutations in the MEFV gene that when homozygous give these classic symptoms, patients with atypical mutations or heterozygous mutations often have a different clinical course. We present identical twin siblings with compound heterozygous MEFV mutations but differing clinical phenotypes.

Case Description: The index patient is a 3 year old girl, conceived via IVF, who began having fevers at age 2.5. Her fevers occurred every 4 weeks for 4 months before she was referred to immunology for evaluation. Her parents describe her as happy and otherwise not ill appearing during these episodes. Genetic testing for Familial Mediterranean Fever revealed compound heterozygous E148Q and P369S mutations in the MEFV Genes. Initiation of colchicine therapy in the affected sibling has resulted in a complete resolution of her symptoms. A trial off colchicine resulted in return of cyclic fevers.

Her identical twin sister was also tested, and carries the same mutation, but is still asymptomatic. This created great concern amongst their parents who had genetic testing prior to undergoing IVF that revealed no parental mutations in MEFV. In consultation with genetics the mother was tested again through the same laboratory that had performed testing on the children. This revealed an identical mutation in mom who is also asymptomatic.

Conclusions: Although classic homozygous MEFV mutations have resulted in well described fever syndromes, there is considerably less data on heterozygous and compound heterozygous MEFV mutations. In these two identical siblings only one patient has a classic manifestation of Familial Mediterranean Fever. While it is possible that the other twin will develop similar symptoms later on in life, it is also possible that another factor is necessary to trigger symptoms in this unusual genetic presentation of FMF. In addition this case highlights the importance of understanding the testing method used by the laboratory performing the genetic testing. While the mother was initially reported as negative the laboratory that performed her testing only tested for the most common MEFV mutations. More complete testing, that included the entire gene sequence, revealed that she did contain an MEFV mutation in E148Q, which although more rare is thought to be pathologic when combined when combined with a second mutation.

(149) Submission ID#811884

A De Novo Variant in AKT3 Associated With Megalencephaly and Immunodeficiency

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Abstract/Case Report Text

There are several lines of evidence that link the PI3K/AKT/mTOR signaling pathway to primary Immunodeficiencies. Hyperactivation of the PI3K/AKT/mTOR/S6K signaling pathway in immune cells can be the consequence of dominant gain-of-function mutations in the genes encoding for PI3Kδ that cause the activated PI3Kδ syndrome (APDS). Patients with these mutations may develop immunodeficiency and immune dysregulation as well as neurodevelopmental delay and growth retardation. In addition, mutations of genes within the PI3K-AKT-mTOR pathway were also known to cause megalencephaly and segmental cortical dysplasia. Mutations in AKT3, a member of the AKT family of proteins and a downstream effector of PI3K-mediated signaling, was shown to be associated with autosomal dominant megalencephaly-associated syndromes.

Here we describe a 4 year old girl, born to consanguineous healthy parents, who presented with megalencephaly, developmental delay, hypotonia, cervical lymphadenopathy and hepatosplenomegaly. The patient had recurrent hospital and ICU admissions for idiopathic thrombocytopenia (treated with IVIG), recurrent laryngitis, recurrent peritonsillar abscess, preorbital cellulitis, conjunctivitis with purulent discharge, otitis media, pneumonia with pleural effusion (required drainage), Metapneumovirus pneumonia with respiratory failure, recurrent skin cellulitis, and abscesses that grew MRSA (required drainage). In addition, the patient is known to have asthma and allergic rhinitis. MRI of the brain showed megalencephaly, ventriculomegally, thin and dysplastic Corpus Callosum, a normal cerebellum, and myelination appropriate for age. Immunoglobulin levels, lymphocyte subsets and the oxidative burst test were all within normal limits. CMV and EBV were not detected. Bacterial cultures grew MRSA (skin), Strept. pneumoniae, H. influenzae, and E. coli (urine). Extensive metabolic workup was done, which was inconclusive (metabolic/mitochondrial diseases). Whole exome sequencing identified an AKT3 variant c.958G>A; p.(Asp320Asn) in exon 10. The variant was identified in the patient but not in the parents and it was confirmed by Sanger sequencing. Further molecular testing concluded that the variant is caused by a de novo mutation during early development.

Although pathogenic variants in AKT3 gene were shown to be associated with megaloencephaly-associated syndromes, no associations with immune deficiency have be reported. Functional studies will be pursued to confirm the link between the clinical phenotype and the identified variant in the AKT3 gene.

(150) Submission ID#811900

Biallelic CARD11 Deficiency Impairs B Cell Development And Function Causing Profound Combined Immunodeficiency And Enteropathy

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Abstract/Case Report Text

Introduction/Background: The caspase recruitment domain family member 11 (CARD11)–B cell CLL/lymphoma 10 (BCL10)–MALT1 paracaspase (MALT1) [CBM] complex is a critical signalling adaptor that regulates lymphocyte activation, proliferation, survival, and metabolism. Primary immunodeficiencies affecting each component (termed 'CBM-opathies') result in broad clinical manifestations ranging from combined immunodeficiency (CID) to atopic disease or lymphoproliferation. We present the laboratory and clinical findings of two Canadian First Nations patients found to be homozygous for the same novel CARD11 mutation (c.2509C>T; p.R837*) causing complete CARD11 deficiency.

Results: We recently identified an 8-month-old boy who presented with a severe case of entero/rhinovirus bronchiolitis with interstitial lung disease and a 17-year-old boy with a history of severe pulmonary infections with bronchiectasis (including PJP), chronic sinusitis, candidiasis, invasive bacteremia, and severe ileo-colitis and oral ulceration requiring total colectomy. Testing of both patients demonstrated absent Tregs, elevated naïve B cells with absent memory B cells, and panhypogammaglobulinemia. Next generation sequencing revealed that both patients were homozygous for the same novel variant of CARD11 (c.2509C>T; p.R837*), which rendered CARD11 protein undetectable by immunoblot. CARD11 deficiency was confirmed by stimulating patient B cells with phorbol 12-myristate 13 acetate (PMA) and ionomycin and immunoblotting for signalling proteins in both the NF-κB (IKKα/β, IκBα, p65) and MAPK (MEK1/2, MKK4, JNK1/2, ERK1/2) pathways as well as cleavage substrates of the MALT1 paracaspase (RELB, CYLD, BCL10, HOIL1). NF-κB and JNK activation were completely absent and MALT paracapase activity was lost. Furthermore, co-immunoprecipitation experiments revealed that CARD11 was required for optimal MALT1 association with BCL10 in response to stimulation. To define the impact of CARD11 deficiency on the B cell transcriptome, RNA-Seq experiments were performed. This revealed an inability to up-regulate critical genes involved in immunity and tolerance (e.g. CD40LG, CTLA4, IL2, IL10), decreased enrichment in cytokine pathways (e.g. IFN-α, IL-6, TGF-β), and decreased enrichment in MALT1-dependent genes. Furthermore, RNA-Seq confirmed the developmental block observed in patient B cells and suggested that B cells were halted at the centroblast to centrocyte transition. Both patients ultimately underwent hematopoietic stem cell transplantation (HSCT), which restored lymphocyte signalling and activation as measured by NF-κB, JNK, and MALT1 paracaspase substrate cleavage.

Conclusions: We have presented the most comprehensive clinical and molecular characterization of human CARD11 deficiency to date. These two cases highlight the crucial role of CARD11 in regulating B cell development, function, and humoral responses, as confirmed by signalling and transcriptomic analyses. Furthermore, HSCT is potentially helpful for these patients as assays performed on post-transplant cells demonstrated restored signalling and activation.

(151) Submission ID#811904

Barriers to Transition of Care from Pediatric to Adulthood in Patients with a Primary Immunodeficiency

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Abstract/Case Report Text

Introduction: Primary immune deficiencies (PID) can have a significant impact on the quality of life of patients and their families. As more patients with PID are surviving to adulthood, the need to monitor them closely and ensure they are transitioned appropriately is even more crucial. We compared the perspectives of pediatric and adult immunologists toward the transition of patients with PID at our institution.

Methods: Pediatric allergy/immunology providers at Lurie Children's Hospital and adult allergy/immunology physicians at Northwestern University both in Chicago, IL completed respective surveys anonymously (www.surveymonkey.com). Questions were derived from the validated 'ATTITUDE' and 'QUARTT' instruments for transition. Respondents were asked to rate their level of agreement on a 5-point Likert scale, ranging from strongly disagree to strongly agree.

Results: Overall, 9 pediatric and 11 adult providers participated (response rate 71.4%). Of total respondents, 55% thought the transition process should be initiated at age 18-20. About 36% of the adult immunologists selected 21 and older, whereas pediatric providers would begin earlier; 33.3% of pediatric providers note they would initiate transition at age 15-17. Both pediatric and adult immunologists agreed that patients should be transferred when the provider felt they were ready (80%) and when they were in stable condition (70%). Both adult and pediatric immunologists selected transfer of complete medical file as a preferred communication method for transition. Other strategies preferred by adult providers were a referral letter with brief summary of medical history (90.9%) and staff meeting with pediatric and adult immunologists (63.6%), whereas pediatric providers would prefer a joint outpatient dedicated transition clinic (77.8%). Pediatric and adult immunologists, patient, parent, and transition liaison were considered the most important active participants in the transition process. The most prominent barriers to a formal transition were unavailability of a transition coordinator or nurse specialists (100%) or of all disciplines of the interdisciplinary team (95%), and limited time (95%). On the other hand, limited demand (too few patients) was strongly rejected as a barrier. All participants agreed during transition patients should be educated about medications and their side effects, their condition and related potential future complications, and symptoms that require seeking health care. Over 95% of participants also agreed that education about how to set up IVIG and further insurance needs were important. All participants agreed that the transition process should include assistance on how to promote the patients' independence and self-management skills, medication management/adherence, and understanding of immunoglobulin replacement and side effects. Other important transition components included knowing how frequently lab draws are required for monitoring (95%) and having a written individualized transition plan (70%). Pediatric providers also thought having an email or telephone help line would be beneficial.

Conclusion: This study adds to the growing body of literature examining attitudes of immunologists toward transition, and it highlights important transition components and barriers. Further work is ongoing to determine the transition needs identified by patients and parents and define markers for successful transfer in order to build a transition policy at our institution specific to immunodeficiency patients.

(152) Submission ID#811989

Immunoglobulin abnormalities are frequent in Adults with non-cystic Fibrosis Bronchiectasis in Cali, Colombia

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Abstract/Case Report Text

Introduction: Bronchiectasis (BQ) is an abnormal and irreversible dilatation of bronchi secondary to repeated cycles of airway infection and inflammation. Predominantly antibody deficiency is the main group of Primary Immunodeficiencies (PID) in adults and had been reported up 10% of subjects with non-cystic Fibrosis Bronchiectasis (NCFB). Hypergammaglobulinemia (IgG level higher than 1,600 mg/dL) had been observed in 9.2 % of NCFB cases (retrospective data). Diagnostic delay and inappropriate management of patients with Predominantly antibody deficiency can lead to irreversible lung damage or even death from serious infections. The effect of hypergammaglobulinemia on NCFB is unknown. Here we present the frequency of immunoglobulin abnormalities (PAD and HyperIgG) in adults with NCFB in Cali, Colombia.

Methods: We present preliminary data of a descriptive prospective study that will include 260 patients with NCFB. Women and men >14 and < 65 years old will be included. All volunteers will be evaluated by a clinical immunologist, complete blood count and serum IgG, IgA, IgM and IgE levels will be determined. According with clinical suspicious, IgG subclasses, anti-pneumococcal IgG response and B cell subpopulations will be performed. The project will be executed in 24 months. Written informed consent has been obtained for all subjects included. This project count with IRB approvals at Universidad del Valle and Hospital Universitario del Valle.

Results: A total of 103 NCFB cases have been included in the study. The mean age was 46.7 years (14-65 years) with a Female:Male ratio 64:39. Moderate-severe dyspnea was observed in 17/103 cases (medical research council –MRC- dyspnea scale 4 to 5). Recurrent pneumonia was found in 33/103 cases (32%).

The main etiologies of bronchiectasis were: Post-Infection 30/103 (29%); Idiopathic 13/103 (12%); Autoimmunity 12/103 (12%); Primary Immunodeficiency 11/103 (10%) Asthma 11/103 (10%); COPD 4/103 (3.8%); Primary ciliary diskinesia 3/103 (3%); Reflux 3/103 (3%) and others. Primary Immunodeficiencies, 10.6% of NCFB cases, were classified as: Predominantly Antibody Deficiency (10cases) including CVID 5 cases, IgM deficiency 2 cases, IgG subclasses deficiency 1 case, selective IgA deficiency 1 case and Hypogamaglobulinemia 1 case. Combined Immunodeficiency (DOCK8 deficiency) 1 case. Interestingly IgG hypergammaglobulinemia was observed in 29/103 cases (28.1%) suggesting humoral immune response deregulation.

Conclusion

To the best of our knowledge this is the first prospective study evaluating the etiology of non-cystic Fibrosis Bronchiectasis (NCFB) in Colombia. Hypergammaglobulinemia and Predominantly antibody deficiencies affect 39% of adults with NCFB in Colombia. Our study reinforced the necessity to evaluate humoral immune response in patients with bronchiectasis.

Conflict of interest: Authors disclosure any potential financial conflict of interest related to this abstract.

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The Mayo Experience Of Early Onset Inflammatory Bowel Disease – A Case Series Of 51 Patients

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Abstract/Case Report Text

Background: Early-onset Inflammatory bowel disease (EOIBD) is defined as IBD diagnosis in children less than 10 years of age. The occurrence of autoimmune disease in children (where it is relatively rare, compared to adults) may be caused by a high-risk predisposition gene (monogenic disorders). Mayo Clinic Children's Center has a unique care model, where a patient who is referred for EOIBD meets with a team of physicians, including gastroenterology, immunology, genetics, and nutrition. We describe our experience of our EOIBD clinic from an immunologic perspective.

Methods: We conducted a retrospective cohort study through EMR chart review of pediatric patients who were referred to our EOIBD program (2011 - 2019). First diagnosis of IBD under the age of 10 was the inclusion criteria. We assessed the presentation, clinical correlates, and immunologic evaluation. Approval was obtained from Mayo's Institutional review board. Data abstraction and analysis was done using the software JMP.

Results: 51 pediatric patients met the inclusion criteria, with 31(61%) males and 20(39%) females. The median age of IBD diagnosis was 5 years (3-7 years range). Median values and the distribution of variables used in the nutritional and immune evaluation were assessed (Fig 1). Nutritional assessment was remarkable for low to low normal hemoglobin and ferritin levels. Vitamin D and Albumin levels were overall within the normal range. Growth parameters indicated that the median BMI percentile was 57 (28-77). With immune and genetic screening, one patient was found to have X linked Chronic Granulomatous Disease (CGD). Immune evaluation of other patients was overall within normal limits. Fecal calprotectin served a reliable non-invasive biomarker for inflammation with the median being 220.5 (62.7-499.3). 41 of these patients underwent GI pathogen panel testing of which 17 (41%) tested negative, four (10%) tested positive for C. diff, and two (5%) others to Shiga toxin-producing E. Coli. It was also noted during the chart review that most patients had poor disease control despite undergoing treatment with various anti-inflammatory and immunosuppressive drugs. The patient diagnosed with CGD underwent bone marrow transplantation. A higher proportion of patients referred to our program in recent years underwent a more comprehensive multispecialty evaluation.

Conclusion: Awareness of monogenic causes of Inflammatory disorders in children has increased in recent years. It is also important to rule out intestinal infections that can act as IBD mimic. Identifying monogenic disorders and other IBD mimics helps with targeted therapy and symptom improvement in these patients who have a difficult-to-treat disease. The group of children with EOIBD, regardless of whether there is an inborn error of immunity, suffers from very high morbidity and a high burden of disease. Comprehensive immune-nutrition assessment of EOIBD patients paves way for further in-depth immunogenic assessments and allows for global management.

Figure 1: Table with reference range, Median values and Range of distribution (25th to 75th percentile) for variables.

	Normal range	Median	25-75 percentile
Hemoglobin	11.6 - 15.0 g/dl	11.5	10.3-12.7
WBC	3.4 - 9.6 x 10(9)/L	8.4	6.4-11.6
ANC	1.56 - 6.45 x	4.2	3.1-6.8
ALC	0.95 - 3.07 x	2.4	1.7-3.2
Platelet	157 - 371 x 10(9)/L	385	292-502
IgA	61 - 356 mg/dl	146	93-262
IgM	37 - 286 mg/dl	80	49-143
IgG	767 - 1590 mg/dl	1050	886-1420
CD45	0.82 - 2.84	2.24	1.5-3.15
CD3	550 - 2202	1610	1151-2127
CD4	365 - 1437	998	737-1400
CD8	199 - 846 cells/mcl	442	304-767
CD19	91 - 409 cells/mcl	421	270-1004
CD16+56+	59 - 513 cells/mcl	174	79-174
Ferritin	11 - 307 mcg/l	16	10 - 64
Vitamin D	20 - 50 ng/ml	32	25 - 40
Albumin	3.5 - 5 g/dl	4.2	3.6 - 4.7
Fecal Calprotectin	<50 mcg/g	220.5	62.7 - 499.3

(154) Submission ID#812007

Chronic Granulomatous Disease and Human Immunodeficiency Virus: A Patient With Dual Primary And Acquired Immune Deficiencies

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Abstract/Case Report Text

Background: Chronic granulomatous disease (CGD) is a primary immunodeficiency (PID) affecting the NADPH oxidase system in phagocytes resulting in increased susceptibility to catalase-positive organisms. Human immunodeficiency virus (HIV) is an acquired immunodeficiency of T helper cells that increases risk for opportunistic infections. Combined impact of CGD and HIV has been rarely reported. In 2001, Sereti and Holland presented the first report of dual impact of CGD and HIV in a patient with disseminated nocardiosis. Because their CGD patient admitted to history of IV drug use, he was frequently screened for HIV.

Case: A 19-year-old African American male with known CGD tested positive for HIV1 by Western blot in the ED in 2009 when he presented with complaints of intermittent fever and cervical lymphadenopathy. His CGD was diagnosed by NBT blood testing at 4 years of age. He had frequent skin infections and fever prior to diagnosis. Clinically, he did so well that his CGD diagnosis was questioned by his immunologists. However, CGD was confirmed by 2 additional abnormal NBT tests and, ultimately, DHR flow cytometry testing.

During his second infectious disease consultation for HIV, at age 19, he disclosed that he was bisexual. Previously, the patient was screened for HIV1 and HIV2 antibodies in 2003 due to anal fissure. He was screened again in 2007 for marked cervical and supraclavicular lymphadenopathy. His CD4+ T cell absolute count was noted to be low (293/mm3) in 2006 at age 17. Until 2009, his prior HIV screenings were negative.

During his CGD treatment course as an adult, he was known to be variably adherent with administration of interferon gamma due to adverse effects, particularly pain at the site of injection and malaise. At the time of his positive HIV Western blot in 2009, his CD4+ T cell count was 237/mm3. After starting HIV antiretroviral treatment, his viral load became undetectable. At age 23, he had Burkholderia cepacia pyelonephritis resulting in left nephrectomy. Sepsis from B. cepacia was fatal (positive blood cultures without known primary source) in 2019 at age 29. His recent viral load was still undetectable and CD4+ count was 240/mm3.

Summary: Our case reveals the complexities of treating a patient with both primary and acquired immune deficiencies. It illustrates the importance of taking a thorough social and sexual history starting in adolescence, including those patients with PID. Patients with PID should be followed closely by a primary care physician, in addition to an allergist-immunologist and infectious diseases specialist, to ensure age appropriate medical and developmental screening. The recognition of PIDs is improving due to better screening, awareness, and treatment. Currently, the rate of HIV infection is highest among young homosexual African American males. It remains important to understand the epidemiology of primary and acquired immunodeficiencies to best identify those at highest risk.

(155) Submission ID#812008

Phosphatase and tensin homolog (PTEN) hamartoma tumor syndrome identified by newborn T cell receptor excision circle screening for severe combined immunodeficiency

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Abstract/Case Report Text

Introduction: Class IA phosphatidylinositol-3-kinases (PI3Ks) are heterodimers with both regulatory (p85α, p85β, p55) and catalytic (p110α, β, or

δ) subunits that are critical for cellular signaling. Heterozygous gain-of-function (GOF) mutations in PIK3CD (encoding p110δ) result in activated PI3K δ syndrome 1 (APDS1), while heterozygous loss-of-function (LOF) mutations in PIK3R1 (encoding p85α) result in activated PI3K δ syndrome 2 (APDS2). Given its role as a negative regulator of the PI3K signaling pathway, heterozygous LOF mutations in PTEN (encoding phosphatase and tensin homolog, PTEN) result in a clinical phenotype that approximates that of APDS1/APDS2 and is therefore referred to as activated PI3K δ syndrome-like (APDS-L). However, sequelae of heterozygous PTEN LOF mutations extend beyond the immune system and include a group of disorders collectively known as PTEN hamartoma tumor syndrome (PHTS). Although severe T cell lymphopenia at birth would be unexpected in APDS1, APDS2, or APDS-L, below normal T cell receptor excision circle (TREC) counts have been reported in APDS1, but only in individuals outside of the neonatal period. Herein, we describe an infant girl with a low TREC count at birth who was found to have PHTS.

Case Description: A 1-day-old girl, born at a gestational age of 39 weeks, was found to have a low TREC count of 22/microliter (normal => 40). A second TREC count obtained at 2 weeks of age resulted as 16/microliter. Arguing against a diagnosis of severe combined immunodeficiency (SCID), flow cytometric analyses performed at 3 weeks of age revealed only a modestly diminished CD3+ T cell count (1599/microliter; 1240 CD4+ and 277 CD8+) with a normal percentage of naïve and memory CD4+ T cells (78% and 22%, respectively). By 7 months of age, her CD3+ T cell count dropped to 828/microliter, which was accompanied by a significantly decreased percentage of naïve CD4+ T cells (56%). Sequencing and deletion/duplication analysis was pursued via a commercially available 207-gene panel aimed at genetically defined primary immunodeficiency (PID), in which no clearly pathogenic mutations were identified. Over the following months, the patient was noted to have macrocephaly, tall stature (99th percentile), axial hypotonia, and gross motor delays. Sequencing and deletion/duplication analysis was then pursued via a commercially available 29-gene panel aimed at genetically defined macrocephaly and overgrowth syndromes, in which a hemizygous pathogenic mutation in PTEN (c.512A>G, p.Gln171Arg) was identified. Subsequent flow cytometric analyses demonstrated findings characteristic of APDS-L, including expanded transitional and CD21lo B cells, decreased isotype switched memory B cells, increased effector memory T cells, a lowered threshold for intracellular calcium mobilization upon B cell receptor engagement, and increased basal Akt (protein kinase B) and S6 (ribosomal protein S6) signaling.

Discussion: We report the first case of PHTS identified by newborn TREC screening for SCID. As PTEN is not included in most commercially available, SCID- or PID-tailored gene panels, PHTS would be missed by conventional genetic testing. Therefore, analysis for variants in PTEN should be considered in neonates with low TREC counts, macrocephaly, developmental delay, and other suggestive sequelae.

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Combination Therapy Targeting Interferon Gamma and JAK-Dependent Signals Abrogates Primary Hemophagocytic Lymphohistiocytosis

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Abstract/Case Report Text

Primary (or familial) hemophagocytic lymphohistiocytosis (HLH) is a rare, life-threatening hyper-inflammatory syndrome affecting mainly young children. It is caused by mutations in genes involved in the granule-dependent cytotoxic pathway, inducing extreme inflammation and massive tissue infiltration by activated T cells and macrophages. Standard chemotherapy-based treatment regimens are toxic and induce remission in only 80% of patients. To this day, HSCT is the only available curative treatment, but the inability to efficiently control the inflammation in many patients prior to transplantation often leads to graft failure, with transplant-related mortality around 25%. Thus, the development of new, more potent and less toxic anti-inflammatory regimens would be a major advancement in the treatment of HLH. Here, we hypothesize that combination therapies targeting several JAK-dependent cytokines will be more effective than monotherapy to reduce the life-threatening symptoms induced by this pathology.

Using a perforin-deficient (PKO) mouse model, we first tested the effects of blocking antibodies against IFNγ, the dominant cytokine secreted during HLH, in combination with antibodies targeting other highly elevated cytokines, such as IL-6 and IL-18, on the manifestations of the disease. We found that anti-IL-6R and anti-IL-18 antibodies, when used in combination with anti-IFNγ antibodies, did not significantly improve the symptoms of HLH compare to anti-IFNγ antibodies alone. Further, we found that targeting the JAK-STAT signaling pathway with ruxolitinib, a specific inhibitor of JAK1 and JAK2, molecules downstream of IFNγ and IL-6, but not IL-18 signaling, was as beneficial as anti-IFNγ monotherapy. Next, we tested the efficacy of ruxolitinib in combination with anti-IL-18 antibody, as this later cytokine is not JAK-dependent and was shown to drive macrophage activation syndrome in other contexts. Unfortunately, this combination did not result in better symptom resolution than the use of ruxolitinib only. In contrast, combination therapy using ruxolitinib and anti-IFNγ antibodies showed a striking synergistic effect on the resolution of most disease manifestations, to such an extent that our PKO mice presented a clinical phenotype indistinguishable than that of a C57BL6 control mice. Our findings demonstrate that JAK-dependent cytokines are the main cytokines driving the progression of HLH in PKO mice. Collectively, our results suggest that anti-IFNγ antibodies and ruxolitinib, although effective independently, should be used in combination to more efficiently suppress HLH progression. These results are particularly relevant since the emapalumab, an anti-IFNγ monoclonal antibody was recently approved by the FDA for the treatment of HLH while ruxolitinib will soon be in clinical trials for this indication. This project was supported by funds from the Fondation de Cancérologie Charles Bruneau and the Canadian Institutes of Health Research (MOP-130469).

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Runs Of Homozygosity Analysis Of Whole Exome Sequencing Data Revealed A Homozygous TCF3 Branch Point Sequence Variant In Consanguineous Brothers With Immunodeficiency

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Abstract/Case Report Text

Introduction: Transcription Factor 3 (TCF3), also known as Transcription factor E2-alpha (E2A), is a helix-loop-helix transcription factor which plays a critical role in lymphopoiesis. TCF3 is required for B and T lymphocyte development. Defects in TCF3 have been associated with Agammaglobulinemia 8, autosomal dominant, characterized by low levels of immunoglobulin and early onset recurrent bacterial infections. Deletion or diminished activity of TCF3 may also play a role in lymphoid malignancies.

Runs of homozygosity (ROH) are contiguous stretches of homozygous genotypes at consecutive polymorphic DNA marker positions. ROH are important reservoirs of homozygous deleterious variation. The homozygosity heterogeneous HMM (H3M2) algorithm was specifically developed for analyzing whole exome sequencing (WES) data.

The branch point sequence (BPS) is an essential splicing signal located ~15-55 bases upstream of splice acceptor sites. While BPS variants are rare, they may result in aberrant pre-mRNA splicing and genetic disorders. These variants may be overlooked by standard WES analysis methods because they are intronic and the mammalian BPS is a degenerate motif.

Objective: Describe the method used to identify a BPS variant in consanguineous brothers with immunodeficiency, including early onset recurrent infections and B-ALL, hypogammaglobulinemia, T and NK lymphocytosis, low B cells, and low naïve T cells.

Methods: WES was performed for all family members. Data were analyzed using standard read mapping, variant calling and annotation methods. ROH were analyzed using the H3M2 algorithm (Magi et al. 2014). ROH from the siblings was intersected (BedTools) and the output was submitted to the Genomic Oligoarray and SNP array evaluation tool (v3.0). Variants were confirmed by Sanger sequencing.

Results: No candidate disease variants were detected in the coding regions, 5' or 3' splice sites, or UTRs for both brothers; however, analysis of intersected ROH revealed a homozygous TCF3 intronic variant within a putative BPS (TCF3 c.1451-18A>T). Sanger sequencing of the mutant cDNA revealed activation of a cryptic splice site.

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Early Humoral Immune Priming Affects Vaccine Responses In Young Infants

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Abstract/Case Report Text

Background: The humoral immune system undergoes a critical period of development in infancy with dramatic shifts in B-cell subsets. Prior to complete humoral immune maturation, infants receive over 20 vaccinations with most vaccines requiring booster administrations to generate immunological memory. Vaccination response is related to both environmental and genetic factors (HLA and non-HLA genes). Heritability for routine childhood vaccines has been shown to range from 38-89%. The genetic component of vaccine response suggests we should be able to predict vaccine response in infants with biomarkers.

Methods: Multi-center study of 93 infants born vaginally at full term and followed through 12 months of life. Cord blood was collected at birth & peripheral blood was collected at 6 and 12 months of life. Six-month collection was 2 weeks post administration of routine vaccinations, while 12-month collection was immediately prior to receiving the 12-month booster vaccines. B cell subsets were analyzed with flow cytometry. Vaccine titers and cytokines were measured via multi-plex ELISA. Study was IRB approved.

Results: Our data confirmed the immaturity of the newborn humoral immune system with a lower overall B-cell abundance, a predominance of naïve B cells, an inability to class-switch and produce IgG or IgA, and a Th2 bias. Maturation was observed over the first year with increasing overall B-cell abundance, frequency of memory B-cells producing IgA, IgG, and IgM, and frequency of plasmablasts. sCD14 levels also increased throughout the first year due to microbial translocation reflecting establishment of the microbiome. Conversely, cord blood contained high levels of BAFF, APRIL, sCD40L, IL-4, and IL-21, with levels decreasing thereafter. All infants displayed evidence of humoral immune system activation after getting 6-month vaccines. Total plasmablast levels peaked 2 weeks after receipt of 6-month immunizations. A decrease in total plasmablasts was evident between 6 and 12 months, although levels remained above those at birth, corresponding with the need for 12-month booster vaccinations to maintain long-lasting immunity. IL-21 and IFN-gamma had a significant positive correlation with memory B cells and plasmablasts at subsequent time points suggesting that these cytokines play a role in B cell differentiation and vaccine response. BAFF and APRIL cytokines were elevated at birth, consistent with germinal center formation and underwent a compensatory decrease thereafter. APRIL & sCD163 levels in cord blood significantly correlated with higher tetanus titers at 12 months suggesting that vaccine response may be predicted by cytokine biomarkers at birth. Response to vaccines was also dynamic with IL-2 levels being significantly correlated with tetanus titers at 2 weeks after receipt of 6 month immunizations. Conversely, sCD40L levels did not correspond to B cell development consistent with a known B cell hyporesponsiveness to CD40L in infants.

Conclusion: Humoral immune development is both predictable and dynamic. Biomarkers in the cord blood, produced by the infant, are predictors of B cell development and vaccine response in infancy.

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A Case of Adult-Onset Immunodeficiency with Anti-IFNγ Autoantibodies in A Previously Healthy Adult with Disseminated Mycobacterium Avium Complex Infection

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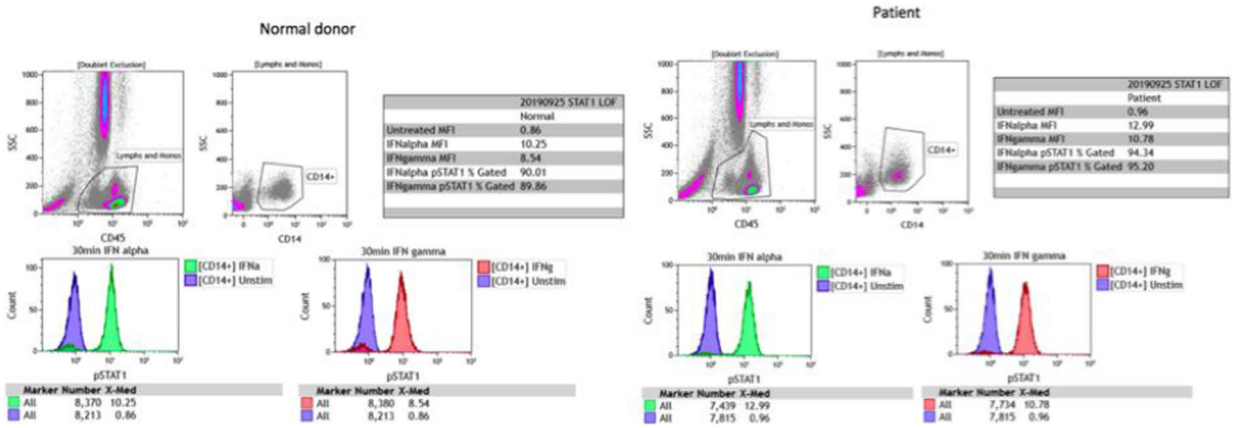
Abstract/Case Report Text

Background: Adult-onset immunodeficiency with anti-IFNγ autoantibodies is a newly described immunodeficiency syndrome characterized by disseminated nontuberculous mycobacterial and other opportunistic infections in previously healthy middle-aged individuals typically from Southeast Asia. It is caused by the presence of autoantibodies directed against the cytokine IFNγ, which is required for intracellular pathogen killing by macrophages as well as phosphorylation of the transcription factor STAT1, which is involved in cell survival gene expression. Successful treatment of the immunodeficiency has been described in prior case reports with immunomodulatory

therapies, including Rituximab. However, there are no standard recommendations for dosing or timing of these agents, or recommendations for long-term monitoring of disease activity.
Case Presentation: A 49-year-old Laotian woman with a history of type 2 diabetes and possible prior Hepatitis C infection presented to the Immunology Clinic for evaluation of immunodeficiency. In the two years prior to presentation, the patient was diagnosed with Mycobacterium avium infection involving the parotid gland and lymph nodes of the neck, Mycobacterium avium complex bacteremia, Histoplasma capsulatum involving the lymph nodes of the neck, and leukocytoclastic vasculitis of the lower extremities. As a child and young adult, she had no severe or recurrent illnesses and did not suffer from any chronic disease. Preliminary immunologic testing demonstrated normal T, B, and NK cell subsets, elevated immunoglobulin G, A, and M levels, and protective titers to tetanus, diphtheria, and 23/23 pneumococcal serotypes. Measurement of anti-IFNɣ autoantibodies was positive, which led to the diagnosis of adult-onset immunodeficiency with anti-IFNɣ autoantibodies. The patient was treated with four doses of monthly Rituximab with resolution of the anti-IFNɣ autoantibodies, restoration of normal STAT1 phosphorylation, and depletion of CD19-positive B cells. After a 4-year period of being lost to follow-up, during which she continued to receive Rituximab every 6 months at the direction of a local provider, the patient re-presented to the Immunology Clinic to re-establish care. At that time, the patient had no evidence of anti-IFNɣ autoantibodies based on titers and normal STAT1 phosphorylation. CD19-positive B cells remained depleted. The patient also confirmed subjective clinical improvement and denied any interim infectious complications.
Conclusion: This case provides an example of successful treatment of a patient with adult-onset immunodeficiency with anti-IFNɣ autoantibodies with Rituximab. It also highlights the utility of IFNɣ functional testing with STAT1 phosphorylation, which may be used to monitor disease activity and to make decisions about ongoing immunomodulatory treatment.



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Immunity after lung transplantation: Detailed monitoring results

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Abstract/Case Report Text

OBJECTIVES: Lung transplantation (LT) is performed using mostly histoincompatible organs and requires strong immunosuppression to avoid transplant rejection. Secondary hypogammaglobulinemia (HGG) is a common post LT complication. Severe HGG in the first-year post-transplant (IgG < 400 mg/dL) occurs in a small percentage of patients. It is associated with recurrent infections. Pre-transplant low IgG predicts post-transplant HGG. However, it does not reliably predict post-LT infections. It is necessary to monitor additional immunological markers to refine the diagnosis of a secondary post-LT immunodeficiency to take preventive measures before infections occur. We sequentially measured T lymphocytes and antibody-mediated immunity in a 67-year-old male receiving a lung transplant for idiopathic pulmonary fibrosis to determine which immune indicators could improve the identification of a secondary immunodeficiency.
METHODS: T and B cell numbers, IgM, IgG, IgA, IgE and IgG subclasses and 13 specific antibodies to S. pneumonia capsular polysaccharides were assessed over a 5 months-long post LT period. The clinical progress, infections and pulmonary function were monitored prior to transplantation and at regular intervals thereafter.
RESULTS: The patient had progressive idiopathic pulmonary fibrosis starting with an episode of pulmonary hypersensitivity 4 years earlier. He developed increasing respiratory failure progressing to complete 02 dependency requiring a LT in June 2019. He was treated with Prednisone, 20mg/day continuously for 3 months, then decreased to 15 mg/day. Other immunosupressants included Mycophenolic acid and Tacrolimus and on/off antibiotics that eventually led to severe tendinitis at 4-5 months post LT. At 4½-month post LT he developed an early onset bronchiolitis obliterans syndrome (BOS) that was controlled by increasing the prednisone dose.
Sequential immunologic evaluation showed his IgG dropping from 1,400 mg/dl to 800 after two weeks and then remaining stable at that level for the rest of the observation period. IgM and IgA had minor variations and IgE remained very low. IgG2 fell from 700-800mg/ml pre-LT to 180-200 in 2 weeks and remained stable at that level thereafter. Antibodies against S. pneumoniae polysaccharides started high, between 5-15 ·g/ml for all 13 serotypes and fell rapidly in the first 2 weeks post LT, then continued a steady decline with > 50% serotypes falling < 1.3·g/ml at 5 months. Twelve of 13 pneumococcal serotype antibodies increased above 1.3·g/ml after IgG replacement at 5 months.
CD4 T lymphocytes decreased from ±1,000 cells/ul to 500-600 at 1 month, remaining at that number after that. CD4/TH17 cells increased from 4–32 cells/ul to 178 at 5 months when prednisone was tapered down and then decreased to 12 after increasing the prednisone dose again. This decrease coincided with a reduction in BOS manifestations.
CONCLUSIONS: Immune monitoring revealed an independent decrease of immunoglobulins with a stronger decrease in IgG2 and specific

pneumococcal antibodies. The role of TH17 cell increase in developing BOS needs further investigation.

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Malignancy in STAT3 Mutated Hyper IgE Syndrome

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Abstract/Case Report Text
Background: Loss of Function (LOF) STAT3 (AD-HIES; Job's Syndrome) caused by dominant negative mutations in STAT3 is a rare primary immunodeficiency characterized by sinopulmonary infections and eczema as well as connective tissue and vascular complications. STAT3 is a frequent target of cancer therapies due to its role in certain malignancies for cell proliferation and metastasis. With LOF STAT3, decreased incidence of some cancers may be expected, however increased rates of lymphoma are described. We sought to describe the incidence and spectrum of malignancy in our relatively large LOF STAT3 cohort.
Methods: We performed a retrospective analysis of 158 LOF STAT3 patients evaluated at the NIH clinical center to determine the type of malignancies diagnosed, treatments received, and outcomes following therapy.
Results: A total of 9 patients with 10 malignancies were identified (cancer incidence 6%). Six patients (4%) were diagnosed with non-Hodgkin lymphoma (NHL); 5 with diffuse large B-cell lymphoma (DLBCL) and 1 with Burkitt lymphoma (BL) with age at diagnosis ranging from 4 years to 66 years with median age of 31 years. Pathology staining for EBV was available in four patients; all of whom were negative by EBER. All 5 DLBCL patients received DA-EPOCH-R for 3-6 cycles, and all achieved complete remission. Five of 6 patients with lymphoma are alive and disease-free. One patient died of heart failure 16 years post chemotherapy without disease relapse. Two patients were diagnosed with papillary thyroid carcinoma at ages 26 and 27, one of whom was subsequently diagnosed with NHL. Two other patients were diagnosed with basal cell carcinoma of the skin at ages 42 and 48; both of whom had prior voriconazole exposure.
Conclusion: Malignancy, most commonly NHL, occurs in patients with LOF STAT3 mutations. NHL should be considered in patients with progressive lymphadenopathy, and thyroid carcinoma should be considered in patients with thyroid nodules. Patients treated with voriconazole are at an increased risk of skin cancer and require careful skin monitoring. As survival increases, it will be important to monitor the incidence of malignancies diagnosed, as it is possible that decreased STAT3 signaling may prove to be protective of some cancers, such as colon and breast carcinoma, in which increased STAT3 signaling is implicated in pathogenesis.

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High Frequency of Primary Immunodeficiencies in Adults with Recurrent Pneumonia in Colombia

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Abstract/Case Report Text
Introduction: Recurrent pneumonia is defined as 2 or more episodes of pneumonia in one year or more than 3 pneumonias throughout life (with radiological resolution between episodes). In retrospective studies, up to 30% of adult subjects with recurrent pneumonia coursed with Primary Immunodeficiencies (PID). Prospective studies evaluating the etiology of recurrent pneumonia are scarce. Diagnostic delay and inappropriate management of patients with PID (predominantly antibody deficiency for example) could lead to irreversible lung damage or even death from serious infections. Here we present the frequency of Primary Immunodeficiencies in adults with recurrent pneumonia in Cali, Colombia.
Methods: We present preliminary data of a descriptive prospective study that will include 100 patients with Recurrent Pneumonia. Women and men >14 and < 65 years old will be included. All volunteers will be evaluated by a clinical immunologist, complete blood count and serum IgG, IgA, IgM and IgE levels will be determined. According with clinical suspicious, IgG subclasses, anti-pneumococcal IgG response and B cell subpopulations will be performed. The project will be executed in 24 months. Written informed consent has been obtained for all subjects included. This project count with IRB approvals at Universidad del Valle and Hospital Universitario del Valle.
Results: A total of 52 recurrent pneumonia cases have been included in the study. The mean age was 38.8 years (14-65 years) with a Female:Male ratio 28:24. Moderate-severe dyspnea was observed in 4/52 cases (medical research council –MRC- dyspnea scale 4 to 5). Non cystic fibrosis bronchiectasis was found in 33/52 cases (63%).
The main etiologies of recurrent pneumonia were: Primary Immunodeficiency 17/52 (32%); Asthma 4/52 (7.6%); Autoimmunity 3/52 (5.7%); Primary ciliary diskinesia 3/52 (5.7%) and others. Hypergammaglobulinemia represented 14/52 (27%) of cases.
Primary Immunodeficiencies, 32% of recurrent pneumonia cases, were classified as: Predominantly Antibody Deficiency (15cases) including CVID 7 cases, IgM deficiency 3 cases, selective IgA deficiency 2 cases, IgG subclasses deficiency 1 case, Hypogamaglobulinemia 1 case and Agammaglobulinemia 1 case. Combined Immunodeficiency: DOCK8 deficiency 1 case and Ataxia Telangiectasia 1 case.
Conclusion: To the best of our knowledge this is the first prospective study evaluating the etiology of recurrent pneumonia in Colombia. Predominantly antibody deficiencies and IgG hypergammaglobulinemia affect 60% of adults with recurrent pneumonia in Colombia. This study allows us diagnosed more than 10 new cases of adult onset PID. Immunological evaluation is critical in the assessment of patients with recurrent pneumonia.
Conflict of interest: Authors disclosure any potential financial conflict of interest related to this abstract.
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Vaccine-Strain Rubella Infection, Hemophagocytic Lymphohistiocytosis, and Autoimmunity in Lysinuric Protein Intolerance

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Abstract/Case Report Text

Introduction: Lysinuric protein intolerance (LPI) is an autosomal recessive metabolic disorder due to pathogenic mutations in SLC7A7. It is distinguished by decreased plasma concentrations and increased urinary excretion of lysine, arginine and ornithine and can present with multiorgan involvement and a spectrum of immune deficiency. We present a five-year-old female with LPI, early-onset juvenile systemic lupus erythematous (SLE), hemophagocytic lymphohistiocytosis (HLH), and granulomatous skin lesions that were positive for vaccine-strain rubella.

Methods: Retrospective chart review was conducted. Laboratory investigations included lymphocyte immunophenotyping by flow cytometry, lymphocyte proliferation to mitogen, quantitative serum immunoglobulins, vaccine titers, autoantibodies, metabolic studies, and genetic evaluation by next generation and whole exome sequencing.

Results: A five-years-old female of mixed Native American and African American race presented at 3 years of age with severe failure to thrive, history of recurrent fevers, joint swelling, recurrent skin lesions, severe anemia and neutropenia, and hypergammaglobulinemia. Upon further evaluation, she demonstrated hyperferritinemia and ANA, RNP, Smith, and SS-A autoantibodies and was diagnosed with early-onset juvenile SLE. Laboratory immune evaluation revealed age-appropriate lymphocyte subpopulations and lymphocyte proliferative responses to mitogens and antigens, markedly elevated IgG, IgA, IgM with no associated monoclonality, and protective tetanus and pneumococcal titers. Given her severe clinical manifestations at an early age, concern for immunodeficiency prompted further genetic evaluation with next generation DCLRE1C sequencing, which was negative, and whole exome sequencing, which revealed two heterozygous mutations in SLC7A7, consistent with LPI. Laboratory metabolic evaluation was also consistent with a diagnosis of LPI. She continued to experience recurrent cutaneous lesions on her upper and lower extremities. Biopsy findings were consistent with a granulomatous lesion and subsequently identified by the CDC to have vaccine-strain rubella infection. Due to recurrent pneumonia and concern for pulmonary alveolar proteinosis (PAP), pulmonology was consulted and eventually confirmed PAP, and she has required home oxygen supplementation. Given her history of recurrent infections and vaccine-strain rubella infection, supplemental IVIG was initiated. Her SLE has been fairly refractory to medical management, including systemic corticosteroids, mycophenolate, rituximab, and cyclosporine. She recently developed HLH at 5 years of age and is currently maintained on canakinumab, mycophenolate, and systemic corticosteroids, yet continues to have SLE and PAP that has been difficult to control.

Conclusion: The range of clinical and immunologic findings in lysinuric protein intolerance has varied widely in the literature. Our patient presented with early-onset juvenile SLE and did not develop HLH and PAP until 2 years after initial presentation. Despite relatively normal cellular immunity by laboratory evaluation, our patient was identified to have chronic infection with vaccine-strain rubella virus, indicating severe T cell dysfunction, and poses challenges for future immunomodulatory treatment.

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Spectrum of Malignancy in MAGT1 Deficiency (XMEN)

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Abstract/Case Report Text

Introduction: X-linked immunodeficiency with magnesium defect, EBV infection, and neoplasia (XMEN) disease is caused by loss-of-function (LOF) mutations in the magnesium transporter 1 (MAGT1) gene. It is a rare X-linked combined immunodeficiency and selective congenital disorder of glycosylation. Clinical manifestations include chronic EBV viremia, recurrent bacterial and viral infections, lymphadenopathy, splenomegaly, autoimmunity, liver and central nervous system (CNS) abnormalities. MAGT1 deficiency was first noted to result in chronic EBV infection and an increased susceptibility to EBV+ lymphomas. We recently recognized Merkel Cell carcinoma at a very young age in two XMEN patients, leading to our review of the malignancies in this cohort.

Methods: We reviewed the records of 25 male patients (22 seen at the NIH) with confirmed hemizygous LOF mutations in MAGT1 for diagnosis of malignancy, therapy, and outcome.

Results: We identified malignancy in 11 patients of 25 with MAGT1 deficiency (44%). Four patients had Hodgkin's lymphoma (HL) (ages 15-29 years), three had Non-Hodgkins Lymphoma (NHL) (ages 7-57 years), one had Kaposi sarcoma (5years) , one patient developed EBER-negative liposarcoma (age 27 years) after receiving chemo and radiotherapy for severe lymphoproliferative disease (LPD) at age 13 years and two had Merkel cell carcinoma at exceedingly young ages (14 and 22 years). All patients had chronic EBV viremia. They all received treatment according to established protocols. Currently, all except for three patients are alive and in remission, including one post-HSCT. Overall malignancy survival of 73%. It is important to note that three patients who did not have malignancy had EBV LPD so severe that it warranted treatment with a malignancy protocol, with one mistaken as having lymphoma.

Conclusion: XMEN immune deficiency, an X-linked glycosylation disorder, is a multisystem disease associated with increased susceptibility to

malignancies. Initially, EBV driven lymphoproliferation and lymphoma was described with XMEN; however, with increasing diagnoses, more malignancies are being recognized. All the recognized malignancies are associated, at least in part, with DNA viruses, including EBV, HHV-8, and Merkel cell virus. Understanding the clinical phenotype and pathogenesis of this disease will improve monitoring and early diagnosis of malignancies for patients with MAGT1 deficiency.

(166) Submission ID#812150

Decision Tree and Random Forest Classifiers to Assist the Clinical Diagnosis of Primary Immune Deficiencies

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Abstract/Case Report Text

BACKGROUND: Primary immune deficiencies (PID) constitute a heterogeneous group of over 400 individually rare congenital diseases that involve genes coding for proteins of the immune system, and which result in increased susceptibility to infection, inflammation, autoimmunity, allergy and cancer. The complexity of the diagnostic task, and the intrinsic biases and limitations of the human mind, can be aided by computational tools. Among the available machine learning approaches, decision tree algorithms select the best node to split based on entropy and information gain; random forests build dozens or thousands of decision trees randomly to improve accuracy and reduce overfitting.
AIM: To implement a machine learning-assisted clinical decision support system for the diagnosis of PID.
METHODS: With a local database of patients with suspected IEI, we built a decision tree using c4.5 DTC, and a Random Forest on Python 2.7 (Jupyter Notebook, SciKit, MathPlotLib, Pandas, Numpy). The database was obtained by conducting an electronic search on MedSys of patients with the term "immunodeficiency" in their electronic medical records, and then hand-picking cases in which a PID had been confirmed or ruled out. It consisted of 234 patients, of which 185 had been diagnosed with IEI. We first split the dataset randomly into training (60%) and testing (40%) sets. The decision tree was tasked with classifying correctly PID or NOT. After running the algorithm in the training set, we evaluated in the testing set through cross-validation.
RESULTS: Accuracy was greater than 80% for the dataset (PID/Not). 0.819 for the DTC with 15 levels. The attribute with the lowest Gini coefficient was Low IgA (0.044). Accuracy for the Random Forest classifier was 0.808 with 25 trees. Feature importance was highest for Lung infection (0.054), High IgG (0.043), Low IgA (0.041), Skin infection (0.046), no isolate (0.057), and allergy (0.043); it was lowest for Consanguinity, High IgM, Central nervous system infection, parasites and no infections. During the random generation of trees, accuracy reached up to 87%.
DISCUSSION: We built two classification models. Decision trees lend themselves more easily to learning and deriving rules of thumb from their sequences. Random forests are more robust and better suited for categoric (as opposed to binary) classification. We next want to develop a chatbot, currently under construction, that will ask relevant questions in optimal sequence, and extract undiagnosed patients with suspected IEI, based on statistical "red flags". We also have preliminary results of this process applied to a USIDNET database with over 3,000 patients, and are also working on Multinomial Logistic Regression and Naïve Bayesian Classifiers for this and other databases.

(167) Submission ID#812164

Intravenous Immunoglobulin Associated With Shorter Length of Stay Among Immunocompromised Inpatients With Acute Viral Respiratory Infections

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Abstract/Case Report Text

OBJECTIVES: Acute viral respiratory infections (AVRI) are associated with significant healthcare resource use and cost. The use of intravenous immunoglobulin (IVIG) may be an effective treatment for immunosuppressed patients and reduce overall healthcare resource utilization. The goal of this study was to assess hospital resource utilization associated with IVIG use among patients hospitalized for AVRI.
METHODS: Using data from the 2011-17 Premier Hospital Database, we identified patients hospitalized with a diagnosis of AVRI [respiratory syncytial virus (RSV), parainfluenza virus, rhinovirus, or metapneumovirus], and who had an immune deficiency (chemotherapy treatment, transplant, primary immunodeficiency disorder (PIDD), specific antibody deficiency, other immunodeficiency, or disorders of the immune or lymphatic systems). Patients receiving IVIG within the first 48 hours were compared to patients who did not receive IVIG at all. Due to the nature of the need to better understand the treatment effect associated with IVIG, we used an Inverse Probability Weight-based Regression Model. Since there were substantially more controls than cases, we randomly drew 5,000 controls. A logistic regression model was developed to adjust for factors associated with the probability of IVIG use within 48 hours of admission. This propensity score was then used to weigh subsequent models to assess length of stay (total and ICU) using negative binomial models and logistic regression for inpatient death.
RESULTS: A sample of 1,927 immunocompromised inpatients were identified, 65 receiving IVIG within the first 48 hours of admission and 1,862 who did not receive IVIG. The IVIG group was older (mean age 54 vs 35, $p < 0.001$), had more antiviral use (40% vs 22%, $p < 0.001$), and had less cancer (40% vs 75%, $p < 0.001$). After adjustment for immunity type (transplant, cancer), RSV, PIDD, age, prednisone, antiviral use, ribavirin use, urban hospital setting, teaching status, intubation and lung disease, patients with IVIG use had 3.24 less days of hospitalization ($p=0.027$) and 1.83 less days in the
ICU ($p=0.003$) than non IVIG users.
CONCLUSIONS: This data analysis suggests that hospital length of stay and ICU length of stay were significantly shorter for immunocompromised patients hospitalized for acute viral respiratory infections who were administered IVIG within the first 48 hours of admission, as compared to patients who did not receive IVIG. It is possible that IVIG use may have an impact on hospital resource utilization and costs. Future prospective studies would help further assess the role of IVIG in patients hospitalized with acute viral respiratory infections.

(168) Submission ID#812176

Association of CD8+ T Cell Senescence and Clinical Histories in CD40 ligand deficiency

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Abstract/Case Report Text

Background: CD40 ligand deficiency is an X-linked combined immunodeficiency associated with opportunistic infections and increased risk of malignancies. Expansion of memory CD8+ T-cells with senescent features is known to be associated with chronic immune stimulation including aging, chronic infection and malignancy. CD8+ T-cell characteristics of CD40L deficient (CD40LD) patients in relation to their clinical history have not been described.

Objective: We studied correlation between CD8+ T-cell senescence with clinical histories of CD40LD patients.

Methods: We analyzed the frequency and phenotypic characteristics of peripheral CD8+ T-cell subsets in four CD40LD patients (5, 28, 33 and 34 years old (yo)) and healthy controls (HCs). T cell excision circle (TREC) counts and telomere lengths of the patients and HCs were measured using quantitative PCR. In-depth analysis of CD8+ T-cells of the 5 yo patient and HCs was done using high-dimensional Cytometer Time of Flight analysis (CyTOF).

Results: Three patients (5, 28 and 34 yo) with histories of recurrent infections and poor compliance with immunoglobulin therapy (IVIG) showed an increased frequency of effector memory CD8+ T-cells with the senescent phenotype compared to age matched HCs. Whereas 33 yo patient with excellent IVIG compliance starting at infancy did not show any senescence phenotypes of the CD8+ T-cells. The telomere length and TREC count of each patient correlated with the degree of CD8+ T-cell senescence and their current ages, respectively. In-depth analysis showed similar expression patterns of molecules related to senescence and cytotoxicity in CD8+ T-cells including CD57, t-Bet, EOMES, Granzyme B and perforin in the 5 yo patient and mid-elderly HCs.

Conclusion: Our findings suggest that prompt diagnosis and compliance with IVIG starting at the infancy may prevent early onset CD8+ T-cell senescence in CD40L deficiency.

(169) Submission ID#812193

Eye Guess It's Just My Eye: A Case of Ocular Immunoglobulin G4-Related Disease Without Systemic Involvement

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Abstract/Case Report Text

Introduction: Immunoglobulin G4-related disease (IgG4-RD) is an immune-mediated fibroinflammatory condition that affects multiple organs. When IgG4-RD is found in the ocular adnexa, the term "IgG4-related ophthalmic disease (IgG4-ROD)" is used.

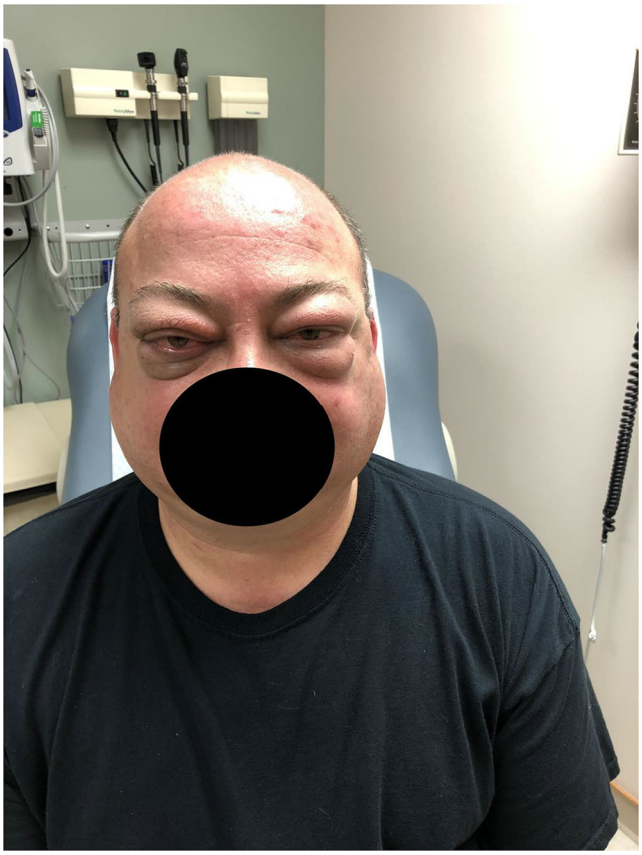
Objective: Our case describes a patient with IgG4-ROD without systemic involvement.

Case: Mr. X is a 46-year-old male with a PMH of CML (on imatinib) and allergic rhinitis who presented to clinic with orbital swelling for twenty years. His swelling had always been responsive to steroids, but would return once steroids were tapered. Patient was diagnosed with biopsy proven CML in 2011 and is currently taking imatinib. Because his peri-orbital edema persisted, a right lacrimal gland biopsy was done which showed "marked lymphocytic infiltrate of soft tissue with lymphoid follicles, many plasma cells, and eosinophils. No atypical histiocytes." Flow cytometry was negative for malignancy.

Results: CRP 4.12 mg/L. ESR 14 mm/hr. IgG4 elevated at 655 mg/dL. CT chest from 2013 and CT chest, abdomen, pelvis from 2019 were without fibrotic changes.

Assessment: When diagnosing IgG4-RD, we categorize diagnosis into three levels (possible, probable, or definite) by three criteria (clinical manifestation, elevated serum IgG4, and histopathology). This is detailed as follows: clinical exam showing organ specific swelling or masses, elevated serum IgG4 (>135 mg/dL), and histopathology with either lymphocyte and plasmacyte infiltration and fibrosis or infiltration of IgG4+ plasma cells (ratio of IgG4+/IgG+ cells ≧40 % and ≧10 IgG4+ plasma cells per high power field). Not all these components are required for diagnosis, but meeting histopathologic criteria makes diagnosis more probable. Our patient's disease was localized to his eye, and patients with IgG4-ROD have unique diagnostic criteria. These criteria are similar to the criteria for IgG4-RD, but emphasize enlargement of the ocular adnexa, less frequent fibrosis, and ≧50 IgG4+ plasma cells per high power field. Our patient's histopathology revealed a lymphoplasmacytic infiltrate, but lacked storiform fibrosis or obliterative phlebitis. His serum IgG4 level was 655 mg/dL, and his biopsy was positive for an IgG4+/IgG+ ratio of 50% and more than 100 IgG4+ plasma cells per high power field. Based on this, he meets criteria for IgG4-ROD.

Conclusion: IgG4-ROD is a rare condition that is usually associated with systemic organ involvement. Our case is unique, as no systemic disease has been detected. We also suspect our patient has been living with IgG4-ROD for several years, as his orbital swelling began in high school. It is important to note that he has been on imatinib, a tyrosine kinase inhibitor, for treatment of his CML. Imatinib inhibits c-abl and platelet-derived growth factor receptor, tyrosine kinases involved in profibrotic pathways. Patient's lack of fibrosis could also be due to his longstanding use of this drug. It is also possible that he has a rare form of IgG4-ROD without systemic involvement. A limited number of such cases have been reported, but no consensus has been made on why disease course was localized. Our patient was started on rituximab, and his serum IgG4 decreased to 295 mg/dL after the first cycle. We hope his disease achieves remission.



Informed consent: Informed consent was obtained from all individual participants included in the study.

(170) Submission ID#812204

Platelet abnormalities with eosinophilia and immune-mediated inflammatory disease (PLTEID), an ARPC1B mutation

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Abstract/Case Report Text

Platelet abnormalities with eosinophilia and immune-mediated inflammatory disease (PLTEID) is a recently discovered combined immunodeficiency with inflammatory and allergic manifestations with few cases reported. We describe a female patient with compound heterozygous mutation in ARPC1B gene with suggestive clinical findings of PLTEID.

A 2-year-old girl presented with chronic diarrhea since neonatal period, with bloody stools and failure to thrive. She also presented atopic dermatitis, recurrent cutaneous and mucosal ulcers, recurrent respiratory infections (4 episodes of otitis media, 2 pneumonias) and many episodes of mucocutaneous candidiasis. Family history revealed a sibling deceased in the second month of life, who presented a similar clinical picture and a paternal uncle and second degree cousin that died in the first year of life. There is no history of consanguinity.

Laboratory evaluation revealed peripheral eosinophilia (1000/mm3), normal platelet numbers with low platelet volume (8,3 fL - reference value 9,4-12,4 fL), normal IgM levels with elevated IgG (1114 mg/dL- RV 453-916 mg/dL), IgA (517 mg/dL - RV 20-100 mg/dL) and IgE (1141 IU/mL - RV A) associated with PLTEID.

The infant receives antimicrobial prophylaxis with sulfamethoxazole-trimethoprim and fluconazole, intravenous immunoglobulin replacement and was referred to hematopoietic stem cell transplantation (HSCT).

This case was the first one described in Brazil and highlights the importance of seeking for a genetic diagnosis in patients with complex clinical phenotypes. Precise diagnosis can impact on treatment approach.

(171) Submission ID#812236

Safety Of Yellow Fever Vaccine And Other Live Attenuated Vaccines In Pediatric Patients With DiGeorge Syndrome

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Abstract/Case Report Text

INTRODUCTION: Patients with DiGeorge Syndrome (DGS) have a variable degree of immunodeficiency due to thymic hypoplasia. Live vaccines are generally contraindicated in patients with combined immunodeficiency (CID). However, in less severe CID, such as partial DGS, those vaccines can be considered depending on the immunologic status of the patient. There are recommendations regarding to measles, mumps, rubella (MMR) and varicella vaccines, but yellow fever vaccine (YFV) is generally contraindicated in this population. Considering the severity of the yellow fever disease and the absence of specific treatment, the use of this vaccine is an important topic for debate in cases of patients from endemic areas.

OBJECTIVE: This study aimed to describe the use of YFV and other live attenuated vaccines in patients with DGS, associating it with their immunological profiles and the presence of adverse effects.

METHODS: Retrospective study of medical records of patients with DGS confirmed by MLPA or FISH, followed in a pediatric reference center for primary immunodeficiencies between 2009 and 2019. Collected data included: demographic characteristics, medical history, history of immunization with live vaccines, postvaccination adverse reactions and immunological profile, including immunoglobulins levels, serologic vaccination responses, lymphocyte immunophenotyping, lymphocyte proliferation responses to mitogens and prophylactic treatments (antibiotic or immunoglobulins).
RESULTS: Thirty-five patients with confirmed DGS and median age of 12 years (2-21y) were included (22M:13F). Thirty-three children (94%) received MMR vaccine: nine presented T lymphopenia. Two of the 9 patients had CD4 < 300, one of them with normal mitogenic proliferation response and the other was not tested. Three of the 33 patients had low immunoglobulins levels (2/33 low IgG, 2/33 low IgM and 1/33 low IgA), and one of them received intravenous immunoglobulin (IVIG). Twenty-nine of 33 had normal serologic vaccination responses. Adverse effect was only reported by one patient, who had one episode of fever after the administration of all vaccines.
Yellow fever vaccine was administrated to 14 children (40%): 2 had T cell lymphopenia (but CD4>500), and another patient had hypogammaglobulinemia and received IVIG and prophylactic antibiotics. Twelve of 14 showed adequate serologic responses to MMR and Hepatitis B. Only 1 patient reported mild reaction (tremors) two days after the YFV administration. The same patient had normal T cells, immunoglobulins and vaccine responses.
Twenty patients (57%) received bacillus Calmette-Guerin vaccine (BCG), 15 (42%) received oral polio, 9 (25%) rotavirus and 8 (22%) received varicella vaccine. No severe adverse events were documented in any patient that received live vaccines, and no patient developed measles, mumps, rubella or yellow fever diseases as a consequence of administration of the vaccine.
CONCLUSIONS: In this cohort of pediatric patients with DGS, YFV and other live vaccines were well tolerated, and no severe adverse events were reported, suggesting that widespread contraindication of YFV may endanger unvaccinated patients with less severe phenotype living in endemic areas. Immunological evaluation and individualized decisions are always recommended, and further studies are needed to assess the safety of the YFV in this pediatric population.

(172) Submission ID#812245

A Phase 1/2 Study of Lentiviral-mediated Ex-vivo Gene Therapy for Pediatric Patients with Severe Leukocyte Adhesion Deficiency-I (LAD-I): Initial Results from the First Treated Patient

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Abstract/Case Report Text
Introduction: LAD-I is a rare inherited disorder of leukocyte (primarily neutrophil) adhesion to endothelial cell surfaces, migration, and chemotaxis resulting from ITGB2 gene mutations encoding for the β2-integrin component, CD18. Severe LAD-I (i.e., CD18 expression on < 2% of neutrophils) is characterized by recurrent serious infections, impaired wound healing, and childhood mortality. Although allogeneic hematopoietic stem cell transplant (alloHSCT) is potentially curative, its utilization and efficacy are limited by HLA-matched donor availability and risk of graft-versus-host disease (GVHD). RP-L201-0318 (clinical trials.gov # NCT03812263) is a phase 1/2 open-label clinical trial evaluating the safety and efficacy of autologous CD34+ cells transduced with a lentiviral vector (LV) carrying the ITGB2 gene encoding for CD18 (Chim-CD18-WPRE) in severe LAD-I.
Methods: Pediatric patients ≥ 3 months old with severe LAD-I (demonstrated by CD18 expression on < 2% neutrophils and at least one prior significant bacterial or fungal infection) are eligible. Peripheral blood (PB) hematopoietic stem cells are collected via apheresis after mobilization with granulocyte-colony stimulating factor (G-CSF) and Plerixafor. CD34+ HSPCs are selected, transduced with Chim-CD18-WPRE LV, and cryopreserved. Myeloablative conditioning with busulfan (therapeutic drug monitoring (TDM) dosing with adjustments to enable target area under the curve (AUC)) is administered over 4 days, followed by infusion of the thawed investigational drug product (RP-L201). Patients are followed for safety assessments including replication competent lentivirus (RCL) and insertion site analysis (ISA), and for efficacy – survival to age 2 (24 months) and at least 1-year post-infusion without alloHSCT, increase in neutrophil CD18 expression, PB vector copy number (VCN), decrease in infections and/or hospitalizations, and resolution of skin or periodontal abnormalities.
Results: An initial LAD-I patient (age 9 years) with recurrent severe infections and documented ITGB2 mutations has been treated as of November 2019. Baseline CD18, CD11a, and CD11b expression were < 1%. Mobilization and apheresis procedures were performed successfully and busulfan conditioning was administered at the target AUC. Investigational product was comprised of 4.2x10e6 CD34+ cells/kg with VCN of 3.8 copies/cell (liquid culture), and was infused without complications. No serious treatment-emergent adverse events were reported. Neutrophil engraftment (3 consecutive days of ANC ≥ 500) was observed 18 days post-infusion. PB PMN CD18 expression 3 months post-treatment was 44.9% with comparable CD11a and CD11b expression levels; PB CD15 (myeloid) VCN at 2.5 months was 1.5. Safety and efficacy data 6 months post-treatment will be available at the time of presentation, in addition to preliminary data regarding a potential additional patient.
Conclusion: Preliminary evidence demonstrates that RP-L201 enables ITGB2 genetic correction with robust CD18/CD11 neutrophil expression in this frequently fatal primary immunodeficiency.

(173) Submission ID#812249

Chronic Glomerulonephritis, Autoimmunity and Recurrent Infections In A Patient With C3 Nephritic Factor

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Abstract/Case Report Text

Introduction: The complement system plays an integral role in the innate immune system and links innate and adaptive immunity. Complement deficiencies, hereditary or acquired, are rare. Acquired deficiencies are more prevalent, occurring in nephrotic syndrome, reduced hepatic synthesis or transiently in sepsis/viremia. They are also seen in the presence of autoantibodies known to cause depletion of complement factors, such as C3 Nephritic Factor (C3NeF). C3 deficiency is associated with infection susceptibility, particularly to encapsulated bacteria, and immune complex disease.

Case Description: A 47 year old male was evaluated for recurrent infection. In childhood, he had recurrent sinusitis, otitis media requiring tympanostomy tube placement and persistent pharyngitis despite tonsillectomy. As a teenager, he developed glomerulonephritis, progressing to end stage renal disease and requiring transplant at age 22. The kidney allograft failed 4 years later, with biopsy demonstrating recurrent glomerulonephritis. The patient was transitioned to peritoneal dialysis and later hemodialysis, due to recurrent PD-related infections. His adult course was complicated by recurrent methicillin sensitive staphylococcal aureus (MSSA) catheter and soft tissue infections (cellulitis and abscess), sinusitis, sepsis (Streptococcal, MSSA and Tularemia), multifocal pneumonia and a left below knee amputation for osteomyelitis that required revision surgery.

Patient reported other autoimmune phenomena including a presumptive diagnosis of vasculitis and possible lupus-like syndrome. The constellation of recurrent infections and autoimmune features was most concerning for an early complement deficiency. Prior work up was notable for low C3, CH50 and AH50 with normal C4, Factor H and Factor I. Extensive laboratory work up revealed normal C1q, C4 level and function, serum immunoglobulins, vaccine titers, Factor B and Factor D levels. Atypical HUS (aHUS) panel revealed a heterozygous silent variant in exon 17 of CFH and a heterozygous polymorphism within an intron in MCP/CD46, seen with increased prevalence in the patient population with aHUS. WES was notable for a variant of uncertain significance in the VCL gene only. C3 level and function were markedly decreased, alongside low CH50 and AH50. Both SC5b-9 level and C3 Nephritic Factor were elevated.

A diagnosis of acquired C3 deficiency due to C3NeF was made and patient was started on Bactrim prophylaxis. He has remained free of serious infection since starting antibiotic prophylaxis.

Discussion: C3NeF stabilizes the alternative pathway C3 convertase, C3bBb, increasing its half-life and blocking dissociation. This leads to unregulated consumption of C3 with subsequent deficiency. C3NeF has been associated with C3 glomerulopathy, infection and partial lipodystrophy. However, there is marked heterogeneity in clinical phenotypes with reported asymptomatic individuals. Our patient's glomerulonephritis likely represents C3 glomerulopathy. Case reports and series of successful treatment of C3 glomerulopathy with rituximab and eculizumab have not commented on immune outcomes beyond the kidney. Other potential therapeutic strategies include plasma cell depletion with either bortezomib or daratumumab. Further study is needed to evaluate these therapies influence on both reversal of C3 depletion and overall impact on immune function in the setting of C3NeF.

(174) Submission ID#812255

Progressive Disseminated Histoplasmosis and Associated HLH: Manifestations of an underlying STAT1 GOF mutation

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Abstract/Case Report Text

Introduction: Patients with heterozygous signal transducer and activator of transcription 1 (STAT1) gain of function (GOF) pathogenic variants exhibit an array of phenotypes including susceptibility to viral, bacterial, fungal and mycobacterial infections, autoimmunity, and cancer predisposition. Progressive disseminated histoplasmosis (PDH) is well-described to affect infants. However, no reports have evaluated underlying monogenic immune dysregulation in previously healthy infants presenting with PDH. We report an infant who presented with PDH and associated hemophagocytic lymphohistiocytosis (HLH) leading to the diagnosis of a heterozygous STAT1 GOF mutation.

Case Report: A previously healthy 9-month old male presented with persistent fever, pancytopenia, transaminitis, elevated ferritin, hepatosplenomegaly and coagulopathy. His clinical and laboratory evaluations were concerning for HLH syndrome. He had no prior history of immune hyperactivation or atypical infections. Secondary causes of HLH were investigated, and patient was diagnosed with PDH based on marked histoplasma antigenemia. Targeted genetic testing did not reveal a genetic etiology of familial HLH. He was successfully treated with a pulse and taper of dexamethasone as well as liposomal Amphotericin B with transition to itraconazole.

Immunologic evaluation at the time of initial presentation demonstrated increased mean channel fluorescence for both perforin and granzyme noted in his NK cells. His NK function was decreased; however, he had a normal CD107a degranulation assay. His B-cell panel demonstrated low non-switched memory B-cells, low switched memory B-cells and low total memory B-cells.

Given his extreme immune activation with histoplasmosis, abnormal immunologic testing, and persistent lymphopenia despite resolution of his infection, a primary immunodeficiency next generation sequencing panel was sent. The results demonstrated a pathogenic variant in STAT1 (c.800C>T; p.ala267Val). This single nucleotide variant has been previously shown to be pathogenic (Clinvar).

The patient was enrolled in the Human Immune Disease Initiative at Vanderbilt University, which allows for immunophenotyping of human leukocytes via CyTOF (mass spectrometry). His evaluation demonstrated relatively increased naïve B-cell populations, and decreased plasmablasts and class-switched memory B-cell populations.

Discussion: The development of HLH is an infrequent manifestation of STAT1 GOF mutations (Faitelson et al, Leiding et al). The mechanism of HLH development in STAT1 GOF is unknown, but possible means include impaired NK cell function as seen in familial HLH (Tabellini et al.) and abnormal IFN-ϒ signaling given the dominant role of this cytokine in driving immune dysregulation in patients with impaired cytoxicity (Ovadia et al.). We suppose that molecular sequencing of STAT1 should be included in patients presenting with HLH. Identification of STAT1 mutations in such patients is critical as JAK/STAT pathway inhibition should be an effective therapy for HLH, potentially avoiding the toxicity of historic agents such as etoposide. Treatment recommendations for STAT1 GOF are not well established. They include ruxolitnib and curative bone marrow transplant, but long-term outcomes with JAK inhibition are lacking and transplant survival

rates to date have been very poor compared to other immune diseases including familial HLH (Leiding et al). As our patient remains healthy on itraconazole, viral and Pneumocystis jiroveci pneumonia prophylaxis without new manifestations of disease, we have deferred these options.

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(175) Submission ID#812256

CTLA4 Mutation: Pathogenic or Not Pathogenic, That Is The Question

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Abstract/Case Report Text

Introduction: Cytotoxic T lymphocyte antigen-4 (CTLA-4) is known to have an important role as a negative regulator of immune responses, participating in the control of regulatory T cells and effector T cells. In mice its absence is associated with fatal autoimmunity and several CTLA-4 mutations, leading to low or absent CTLA-4 expression, have been shown in humans to be associated with a phenotype that includes hypogammaglobulinemia (with recurrent respiratory infections) and several manifestations of autoimmunity (enteropathy, granulomatous lymphocytic interstitial lung disease, organ infiltration, splenomegaly, autoimmune cytopenias, lymphadenopathy, amongst others), in an autosomal dominant mode of transmission. One of the published mutations, c.C257T, that results in an alanine to valine substitution (p.A86V), with a highly conserved alanine at that position, had a CADD score of 24 and was associated with the phenotype above, and was shown to be associated with a low expression of CTLA-4 on regulatory T cells and with low CTLA-4 function (reduction of CTLA-4-mediated transendocytosis).

Methods: After IRB approval, we searched for CTLA-4 mutations present in the BioMe BioBank· biorepository, containing whole exome sequencing data on 30845 patients, with data obtained using Illumina· v4 HiSeq 2500 sequencing platform. Sifting through all the CTLA4 mutations in the data, we identified four patients with the c.C257T mutation described above. Extensive chart review of the four patients was performed.

Results: Four patients were found with the CTLA-4 c.C257T mutation. None of them had any of the described phenotypical characteristics of CTLA-4 deficiency. Patient 1 is a 68-year-old male with history of coronary artery disease, atrial fibrillation, stroke, hypertension, brain aneurysm, chronic kidney disease, gout and depression. Patient 2 is a 29-year-old female with history of morbid obesity. Patient 3 is a 51-year-old female with history of hypertension, obesity, pre-diabetes, dyslipidemia and iron deficiency anemia. Patient 4 is a 70-year-old female with history of peripheral artery disease, hypertension, dyslipidemia, chronic kidney disease and lung cancer.

Conclusion: Prior literature has attempted to characterize the clinical penetrance of CTLA-4 mutations, suggesting it to be around 67%, with that number applying to 45 different mutations in 133 CTLA-4 mutation carriers. We screened a large biorepository of more than 30 thousand patients for CTLA-4 patients and identified four patients that carry one of the best described CTLA-4 mutations, previously validated from a functional standpoint and associated with a severe phenotype. None of the four patients demonstrated any of the previously described phenotypical characteristics, and all four have ages above the median age of onset of 11 years. With the increasing use and broad population application of genetic studies, it is crucial to define the value of identifying presumed pathogenic variants in the absence of the adequate phenotype, with all the prognostic, therapeutic and ethical considerations it may imply.

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Successful Allogeneic Hematopoietic Stem Cell Transplantation for Management of Multiple B-Cell Malignancies in A Patient with Primary Intestinal Lymphangiectasia

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Abstract/Case Report Text

Introduction: Primary intestinal lymphangiectasia (PIL) is a rare disorder characterized by dilated intestinal lacteals resulting in leakage of lymph into the intestinal lumen, causing lymphopenia and hypoproteinemia. For reasons that are incompletely understood, affected patients are predisposed to developing B-cell malignancies with gastrointestinal or extra-intestinal localizations.

Prior case reports of PIL patients with B-cell malignancies have discussed treatment regimens with chemotherapy, radiation, and/or surgery, but neither the use nor the outcomes of allogeneic hematopoietic stem cell transplantation (HSCT) in the management of recurring B-cell malignancies have been readily reported.
Case Description: A 21-year-old man with PIL and an accompanying history of lymphopenia, hypoproteinemia, hypoalbuminemia, and hypogammaglobulinemia was diagnosed with diffuse large B-cell lymphoma (DLBCL) of the liver following a preceding history of Burkitt lymphoma of the ileum at 6 years of age and DLBCL of the liver at 16 years of age, in which each malignancy was genetically distinct. In addition, the patient had a history of benign nodular adenomatoid hyperplasia of the thyroid at 17 years of age that required a hemi-thyroidectomy. Treatment considerations for the patient included chimeric antigen receptor T-cell therapy, autologous HSCT, and allogeneic HSCT, in which allogeneic HSCT was ultimately pursued. Prior to HSCT, the patient was lymphopenic (670 cells/microliter) with significant T-cell lymphopenia (290 cells/microliter) and an increased proportion of memory T-cells (67% of his CD4+ T cells were CD45RO+), as well as hypogammaglobulinemic (IgG 296 mg/dL; IgA 47 mg/dL; IgM 62 mg/dL). Immediately following treatment of his DLBCL with rituximab, ifosfamide, carboplatin, and etoposide, the patient underwent a matched-related sibling donor HSCT with a preparative regimen of busulfan, thiotepa, and fludarabine. Now 18 months status-post HSCT, the patient has maintained full-donor chimerism and has no evidence of graft-versus-host disease or malignancy. As expected, HSCT has not corrected abnormalities in certain parameters associated with his PIL, as he continues to display significant hypoproteinemia, hypoalbuminemia, and hypogammaglobulinemia, but he has an improved lymphocyte count (1,530 cells/microliter).
Discussion: There is no definitive or curative treatment for PIL; furthermore, the genetic etiology of PIL remains unknown. Supportive regimens to help mitigate or offset manifestations of PIL exist, such as adherence to a low-fat diet with medium-chain triglyceride supplementation, but there are no therapies available to prevent or reduce the risk of developing B-cell malignancies in this patient population. Although previous case reports have detailed successful treatment of B-cell malignancies in PIL patients with chemotherapy, radiation, and/or surgery, there are no published consensus guidelines regarding management of B-cell malignancies in the setting of PIL, especially if recurrent in nature. For non-PIL patients with chemotherapy-refractory disease, or recurrent disease following autologous HSCT, allogeneic HSCT is a potentially curative option. Herein, we describe a PIL patient with a history of multiple B-cell malignancies who underwent a successful allogeneic HSCT, indicating that allogeneic HSCT may be an effective treatment option for similarly affected patients.

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DGAT 1 Deficiency Presenting With Intractable Diarrhea, Severe Anemia and Profound Hypogammaglobulinemia

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Abstract/Case Report Text
Introduction Diarrhea in young infants is common and generally self-limited. In persistent cases, the differential diagnosis is broad and includes infections, food protein-induced allergic proctocolitis, congenital diarrheas and enteropathies. In addition to monogenic inflammatory bowel diseases, many cellular, humoral, and combined immunodeficiencies should be considered, including but not limited to CVID, IPEX and IPEX-like phenotypes, LAD, dyskeratosis congenita, intestinal lymphangiectasia, Omenn syndrome, cartilage hair hypoplasia, CGD, IL-10 axis defects, AID deficiency and Wiskott-Aldrich Syndrome.
Case Presentation
A full term infant born after an uncomplicated pregnancy to non-consanguineous Honduran parents presented with non-bloody, non-bilious vomiting and dehydration at 21 days of life. The infant later developed frequent loose stools, some of which were bloody, and failure to thrive. His family and prior medical history, including newborn screen, were normal.
An extensive workup was initiated which showed:
- Persistent and severe anemia with a hemoglobin nadir of 3.5 mg/dL
- Hypoalbuminemia requiring multiple infusions
- Elevated alpha-1-antitrypsin and calprotectin level in stool
- Profound hypogammaglobulinemia with normal IgA, IgM and IgE for age
- Normal gross and histologic findings on esophagogastroduodenoscopies and colonoscopies besides a gastric ulcer thought not be the cause of his anemia
- Normal abdominal imaging including ultrasound, CT angiography and MRI
- No source of bleeding on Meckel scan or exploratory laparotomy
- Normal DHR assay, G6PD level and positive myeloperoxidase stain
- Immunophenotyping showing T cell lymphocytosis affecting CD4+ more than CD8+ compartment, with normal lymphocyte proliferation to mitogens
- Normal sweat chloride level

Genetic testing was initiated with a targeted immunodeficiency panel which showed variants of unknown significance in ADAR, DOCK8, LYST, PTPRC and TBX1 genes, none of which adequately explained his presentation. Whole exome sequencing showed that he was a compound heterozygote in the DGAT1 gene. A pathogenic variant c.751+2T>C (IVS8 + 2T>C) was inherited from the father and a likely pathogenic variant c.1073G>C (p.R358P) was inherited from the mother. Patient was diagnosed with DGAT1 deficiency, an inborn error of lipid metabolism resulting in protein-losing enteropathy (PLE). Under gastroenterology's guidance, a low fat diet was initiated, resulting in rapid improvement in stool consistency, weight gain, albumin level and stool alpha-1-antitrypsin level. He remains on subcutaneous immunoglobulin replacement therapy for ongoing hypogammaglobulinemia.
Conclusion: Protein-losing enteropathies commonly present with intractable diarrhea and significant laboratory derangements due to malabsorption including hypogammaglobulinemia. As a result of these findings and since many of the etiologies are immunologic in origin, immunologists are an integral part of the evaluation of such cases. In cases where immune system interrogation reveal normal results, genetic testing is crucial in guiding the diagnosis. In our case, whole exome sequencing not only provided the diagnosis but also characterized a variant that was previously of unknown significance as likely pathogenic. Dietary management provided rapid improvement in growth and nutritional status. Ongoing monitoring will reveal if this management also assists in IgG level maintenance and hematologic abnormalities or if even more stringent control of dietary fat will be required

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Immune Dysregulation Polyendocrinopathy X-Linked (IPEX) Symptomatology With A Hemizygous Variant In The Non-Coding Polyadenylation (polyA) Signal of FOXP3

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Abstract/Case Report Text

Background: FOXP3 gene mutations are associated with immune dysregulation polyendocrinopathy X-linked (IPEX) syndrome, a rare X-linked monogenic disease of immune dysregulation and autoimmunity. The classic presentation consists of severe enteropathy, dermatitis, and endocrinopathies (commonly early onset insulin dependent diabetes mellitus). Clinical presentation and severity can be variable even in family members with the identical variant. We present a patient with IPEX symptomatology and a hemizygous variant in the polyadenylation (polyA) signal of FOXP3 that is classified as a variant of uncertain significance (VUS). This specific variant was reported in a single case study in which the patient improved after hematopoietic stem cell transplantation (HSCT).

Case Presentation:

A 2 month-old ex 34-week gestation boy was admitted with lethargy, hypovolemia, electrolyte disturbances, and acute kidney injury. He developed persistent diarrhea and vomiting after receiving Rotavirus vaccine. His family history is significant for early deaths of three maternal uncles - one stillborn, one death at 6 months and another at 2 years from unknown gastrointestinal problems.

He demonstrated peripheral eosinophilia (to 4.26 K/uL), elevated IgE, and anemia requiring multiple transfusions. He developed severe enteropathy with hypoproteinemia requiring total parenteral nutrition, continual albumin infusions and maintenance of NPO. He had generalized edema, respiratory distress requiring high flow nasal cannula, and repeatedly spiked fevers with negative infectious evaluation. Acute kidney injury improved but renal ultrasound showed persistent nephrocalcinosis. Endoscopy yielded biopsies demonstrating duodenitis with severe villous atrophy, scanty isolated intraepithelial eosinophils and neutrophils, a few crypts with mucin, reactive epithelial changes and increased lamina propria eosinophils. Colon biopsies showed mucosa with focally increased lamina propria eosinophils with scanty neutrophils and surface epithelium without cryptitis. Esophagitis with reactive epithelial changes, spongiosis, and many intraepithelial eosinophils was also present.

The patient's lymphocytes showed unremarkable proliferation to PHA and PWM. CD4+ CD25+ T cells demonstrated intracellular FoxP3 expression by flow cytometry. A commercially-available immunodeficiency targeted panel revealed that he was hemizygous for a VUS in FOXP3 (Exon 12, c.*878A>G non coding). This variant is also referred to as an AAUAAA>AAUAAG or AATAAA>AATAAG change in the polyA site. He was also heterozygous for VUS at these additional loci: CD79A c.341C>T (p.Ser114Leu), DOCK8 c.5296C>T (p.Arg1766Trp), MOGS c.1484G>A (p.Arg495Gln), NOD2 c.2110C>T (p.Gln704*), NOD2 c.2110C>T (p.Gln704*), NOD2 c.2110C>T (p.Gln704*).

Discussion: The polyA signal AAUAAA, located 798 bp downstream from the stop codon of the FOXP3 gene, triggers polyadenylation of the mRNA, which is crucial for stabilizing and transport of mRNA. Our patient has a point mutation in this sequence. While currently classified as a VUS, Dorsey et al. (2009) reported the same variant in an IPEX patient in whom clinical symptomatology, including enteropathy, resolved post-HSCT. Unlike our patient, the case report patient had absent FOXP3 expression pre-HSCT. Gambineri et al. (2018) identified mutations in the FOXP3 polyA site in up to 12% of their cohort of 173 patients indicating this is not an uncommon area of IPEX-associated mutation. Given the clinical presentation and the available evidence, we suggest that this mutation is likely pathologic in our patient.

(180) Submission ID#812316

Immune Reconstitution Inflammatory Syndrome or Atypical DiGeorge Syndrome (DGS) Following Cultured Thymus Tissue Implantation for Complete DGS

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Abstract/Case Report Text

Introduction: Implantation of allogeneic cultured thymus, partially depleted ex vivo of T cells, can result in naïve T cell development in patients with complete DiGeorge syndrome (DGS). In a few patients, early and transient skin rash, often characterized as "atypical DGS" or late autoimmune manifestations have been reported following implantation. Here we describe a patient with complete DGS who developed immune reconstitution inflammatory syndrome (IRIS) or atypical DGS following thymus implantation.

Case description: A female patient was diagnosed at birth with complete DGS due to absent T cell receptor excision circles (TREC), hypoplastic thymus, profound hypocalcemia with hypoparathyroidism and cardiac defects. The patient also had microretrognathia, oral motor dysfunction, sialorrhea, recurrent aspirations and reflux requiring a gastro-jejunum feeding tube, low-set ears with right ear microotia, semicircular canals atresia, alopecia and mal-rotated kidneys. Prior to thymus implantation, the patient was thriving, had no skin rash, no eosinophilia and no T cells. Detailed genetic analyses, did not reveal a cause for her syndrome. At 9 months of age pulmonary aspergillosis was diagnosed presumptively. At 10 months of age the patient received an allogeneic T-cell depleted thymus implant from a male donor, without prior conditioning or post-implantation immune suppressive medications. The procedure was uneventful and the patient returned home after 7 days.

Results: Four months after implantation, a pruritic maculopapular rash appeared on the head and trunk that spread to the extremities

including the palms and soles. There was no lymphadenopathy or splenomegaly. An infectious etiology could not be found. Eosinophilia and an increase in liver enzymes were noted. There was an increase of CD4+ and CD8+ T cells with predominantly memory phenotype, which had been undetectable 1 month earlier. Analysis of T cell diversity showed a restricted repertoire with expansion of two V-beta families. There was no evidence of donor cells to suggest graft versus host disease. Skin biopsy showed minimal superficial perivascular inflammatory infiltrate composed mainly of CD163+ histiocytes and rare CD3+ T cells. The patient was treated with prednisone and cyclosporine. A liver biopsy was performed 3 weeks after initiation of treatment that showed moderate and diffuse peri-portal ductular reaction but no duct associated lymphocytic infiltrate or significant duct epithelial injury or ductopenia. The skin rash rapidly resolved with desquamation, while the liver enzyme abnormalities persisted for two more months. Cyclosporine and prednisone were weaned over 2 months. T cell numbers, their response to stimulation and diversity have since normalized, as well as TREC and naïve T cell production. The patient is producing appropriate antibodies to protein and polysaccharide vaccines. Sixteen months after implantation the patient developed Grave's disease with markedly elevated free-T4, undetectable TSH and elevated antibodies to the thyroid receptor, which rapidly normalized with ongoing methimazole treatment. The patient is currently 30 months after the implantation and is free of infections, thriving and developing appropriately.

Conclusions: This patient developed atypical DGS or IRIS, often associated with autologous and allogeneic hematopoietic stem cell transplants, organ transplants or effective treatment of HIV, after successful thymus implantation for complete DGS.

(181) Submission ID#812317

Defective Glycosylation Leads To Impairment of gp130 Wxpression and gp130-Dependent STAT3 Signaling in PGM3-Deficient Patients

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Abstract/Case Report Text

Congenital disorders of glycosylation are a rare group of genetic disorders due to defects in protein glycosylation. Phosphoglucomutase 3 (PGM3) is an enzyme necessary for the synthesis of Uridine Diphosphate N-Acetylglucosamine, an important precursor for protein glycosylation. Patients with autosomal recessive PGM3 deficiency have a multisystemic disorder characterized by a neurologic impairment and clinical features classically observed in autosomal dominant hyper-IgE syndrome due to STAT3 mutations; including recurrent pneumonias, skin abscesses, elevated levels of IgE, and abnormalities in connective tissues and bones. We hypothesized that gp130, a highly glycosylated protein and co-receptor of the cytokine IL-6, would be weakly expressed on PGM3-deficient cells, due to impaired glycosylation.

We studied 6 PGM3-deficient patients from 3 kindreds and showed that IL-6-driven STAT3 phosphorylation was impaired in their PBMCs and EBV-transformed B cells. Accordingly, the induction of SOCS3 target gene was significantly decreased. In contrast, the patients had normal STAT3 phosphorylation and SOCS3 induction downstream of IL-10, a cytokine whose signaling is independent of gp130. Flow cytometry and immunoblotting showed significantly lower gp130 expression in peripheral T-cells and EBV-transformed B cells from PGM3-deficient patients compared to healthy donors. We did also show that in vitro inhibition of N-glycosylation, using tunicamycin in EBV-transformed B cell line from healthy donor, alters gp130-mediated signaling.

Collectively, our findings demonstrate that defective glycosylation in PGM3-deficient patients results in reduced expression of gp130 and consequently, impaired gp130 dependent STAT3 phosphorylation and defective IL-6 signaling. This may account for the overlapping clinical features shared by PGM3 and STAT3 deficient patients.

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Outcomes Following the Use of Intravenous Immunoglobulin Versus Non-Intravenous Immunoglobulin Therapy In The Treatment Of Hospitalized Patients With Respiratory Infections

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Abstract/Case Report Text

Introduction: There are no known effective therapeutic modalities for patients hospitalized with moderate to severe acute viral respiratory infections, and treatment is primarily supportive. Intravenous immunoglobulin (IVIG) has been reported in limited cases to be used in this setting, especially in immunocompromised patients. The primary objective of this retrospective study is to compare clinical and economic outcomes among immunocompromised patients hospitalized with viral respiratory infections who received IVIG to those who did not receive IVIG at a large academic center hospital.

Methods: We performed a double-center, retrospective cohort study of all immunocompromised patients who were hospitalized for acute documented respiratory viral infections between 2011 and 2016. We divided patients into two groups: those who received IVIG therapy for respiratory infections, and those who did not receive IVIG therapy. Data on age, gender, immune status, viral type, immunosuppression type, respiratory support, microbiological data, length of hospital stay (LOS), ICU LOS, as well as death and readmission rates were extracted from medical records. In order to adjust for severity bias typically present in observational data such as these, we employed inverse probability weighting (IPW) using all collected baseline covariates. Outcomes (death, length of stay in hospital and ICU, readmission) were examined using a series of logistic and Poisson regression models adjusting for baseline covariates and employing IPW.

Results: A total of 282 individual hospital admissions were analyzed; 99 patients received IVIG and 183 did not receive IVIG. There were no significant differences between the two groups in terms of mean age, gender . Average age was 40.3, 50% were

female, 74.5% were transplant patients of which 26.6% had lung transplant, 26.6% had liver transplant, 23.1% had bone marrow transplants (BMT), 8.5% had kidney transplant, 7.8% had heart transplant and 4.3% had both solid organ and BMT. 32.3% of patients had a hematologic malignancy, and 2.5% had a primary immunodeficiency. The most common isolated respiratory virus was rhinovirus (51.4%), followed by RSV (25.9%), Parainfluenza (11.4%) and Metapneumovirus (10.6%).
Overall, the use of IVIG as associated with a significantly shorter ICU length-of-stay, with an (OR=-2.46, p=0.001), and a higher hospital readmission rate. In the sub-analysis of patients who received IVIG within the first 48 hours of hospitalization (n=39), IVIG use was associated with a significantly shorter ICU LOS (OR=-6.01, p=0.0), significantly shorter overall hospital LOS (OR=-4.341, p=0.008), and no significant change in readmission rate.
Conclusions: To our knowledge, this is the first retrospective cohort analysis evaluating the effect of IVIG in immunocompromised patients hospitalized with respiratory viral infections. The results suggest that immunocompromised patients receiving IVIG may have a shorter hospital and ICU LOS, especially if IVIG is provided within the first 48 hours of admission. This may result in reduced healthcare costs. This study is limited by its retrospective nature, and the potential bias that patients treated with IVIG are sicker to start with. Future prospective studies are suggested to further evaluate these findings.

(183) Submission ID#812340

Recurrent Stevens-Johnson Syndrome Associated With Mycoplasma Infection In A 15 Year-Old Female Patient

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Abstract/Case Report Text
Introduction/Background: Stevens-Johnson syndrome (SJS) is an immune-mediated disease characterized by extensive epidermal necrosis and detachment resulting in mucocutaneous complications (1, 2). The most common precipitant in children is medication, followed by infection (2,3). Although a clear association between Mycoplasma pneumoniae and SJS has been established, there is a scarcity of literature exploring the role of this infection in recurrent SJS in children (2-4).
Case presentation: A 15-year-old female with prior history of SJS was admitted for mucosal and skin lesions in the setting of community acquired pneumonia. Her past medical history included SJS with eye involvement, secondary to Mycoplasma pneumoniae (Ig M positive), occurring five years prior to this admission. She also had frequent episodes of acute otitis media and sinusitis in early childhood. Family history was negative for immunodeficiency. Her clinical presentation included respiratory symptoms and fever for 8 days treated with ceftriaxone, followed by cefdinir and levofloxacin. Her fever improved the day prior to admission, but she developed conjunctival injection, ocular pain, and ulcerative lesions in her mouth and nares. On physical examination, she had low grade fever with mucosal lesions including conjunctival erythema with serous discharge, painful blisters and denudated skin in lips, perioral area, nares, tongue and oropharynx. Initial testing included negative blood HSV PCR, blood culture, rapid antigen testing for group A Streptococcus and Influenza A/B, and elevated CRP in 4.4mg/dl and ESR 55mm/hr. Right lower lobe pneumonia was confirmed with a chest radiograph. Nasopharyngeal PCR and serum IgM were positive for Mycoplasma pneumoniae. She had a mildly elevated Anti-cardiolipin IgM (17 MPL), a mildly decreased C3 (75 mg/dL) and a negative ANA. She was diagnosed with recurrent SJS secondary to Mycoplasma pneumonia infection. She completed treatment with levofloxacin for Mycoplasma pneumonia, and received cyclosporine and high-dose methylprednisolone. She had bilateral amniotic membrane transplantation to prevent corneal ulceration . She was discharged after clinical improvement, and recurrent oral lesions were noted at followup. Immunological work up as an outpatient revealed normal serum immunoglobulins, normal lymphocyte subsets and low pneumococcal titers with adequate response post-vaccination.
SJS secondary to Mycoplasma pneumonia infection has predominance of mucosal involvement over rash, which was observed in our patient (1, 2). Some case series reported a recurrence of SJS up to 18% within a 7-year follow up. Almost half of patients with recurrent SJS developed multiple sequelae (2, 3). Early diagnosis of SJS, especially in those with prior history of SJS, helps to provide appropriate supportive care, monitoring of complications and treatment of possible superinfections (5,6).
Conclusions:
There is limited information in the literature regarding the role of Mycoplasma pneumoniae associated recurrent SJS in children. It is possible that these episodes are triggered by and/or immune predisposition. There is ongoing discussion regarding whether these clinical presentation should be labeled SJS secondary to Mycoplasma pneumonia infection or, depending of the skin involvement, M. pneumonia-associated mucositis (MPAM) and M. pneumonia-induced rash and mucositis (MIRM) (5,6,7). Mycoplasma should be treated appropriately in patients with recurrent SJS.

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Dual NFKB1 Loss-Of-Function and CXCR4 Missense Variant Presents As Hypogammaglobinemia, Neutropenia, and HPV Susceptibility

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Abstract/Case Report Text

Background: Growing access to genetic testing has facilitated the genetic evaluation of primary immunodeficiencies but has also greatly increased the number of variants of uncertain significance (VUS) encountered in clinical practice. Interpreting the significance of VUS requires multiple lines of evidence.

We describe a neutropenic index patient with hypogammaglobulinemia, unusual HPV susceptibility, and dual heterozygous pathogenic loss-of-function NFKB1 and heterozygous missense CXCR4 VUS. Family analysis showed the NFKB1 variant was inherited from his mother, while the novel CXCR4 variant was present in his father and sister. All four patients presented with recurrent infections, warts, and hypogammaglobulinemia. (Figure 1)

The NF-κB1 gene encodes p50/p105 transcription factor of the canonical NF-κB pathway, the most common autosomal dominant monogenic cause of common variable immunodeficiency (CVID).

CXCR4 is a G-protein-coupled chemokine receptor with CXCL12 as cognate ligand. Autosomal dominant pathogenic gain-of-function CXCR4 variants lead to impaired receptor downregulation and retention of neutrophils and other leukocytes in the bone marrow defining WHIM (warts, hypogammaglobinemia, infections, and myelokathexis) syndrome. All CXCR4 pathogenic variants truncate the carboxyl-tail of the CXCR4 receptor, a region responsible for receptor internalization, with the exception of one missense non-truncating variant p.E343K.

Case series: The proband (P1) is a 19-year-old male with history of recurrent bacterial respiratory tract infections, warts, moderate neutropenia, thrombocytopenia and hypogammaglobulinemia requiring immunoglobulin replacement therapy (IgRT). Bone marrow biopsy didn't show myelokathexis. Next-generation panel sequencing identified a novel heterozygous missense CXCR4 (c.1022C>A, p.S341Y) VUS. The serine residue is highly conserved up to zebrafish. This variant was present in heterozygous form in two cases in gnomAD database (277,984 alleles). Additional whole-exome sequencing revealed a heterozygous pathogenic NFKB1 variant (c.980dup, pA328Sfs*12) located in the N-terminal Rel homology domain, consistent with NFKB1 loss-of-function.

Both, the patient's sister (P2) and their father (P3), carry the heterozygous CXCR4 VUS but not the pathogenic NFKB1 variant, and have history of warts, hypogammaglobinemia, and recurrent infections. The HPV susceptibility is particularly striking in P3 manifesting by genital warts and HPV-positive oropharyngeal cancer. Bone marrow evaluation didn't identify myelokathexis in P2 (P3 is pending).

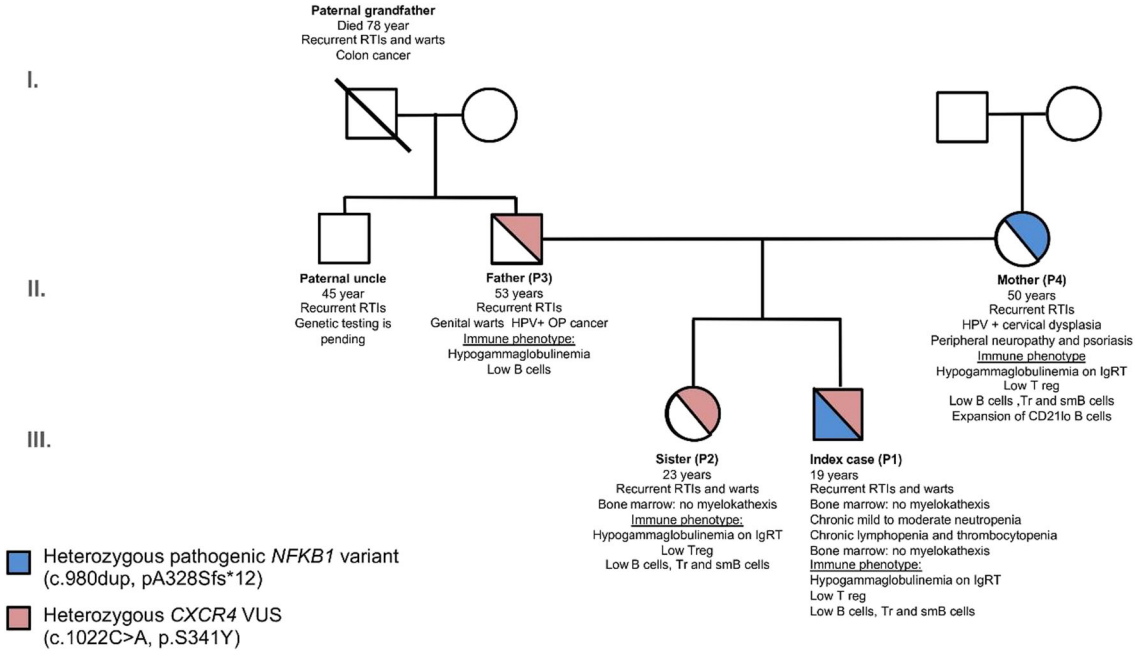
The mother of the index case (P4) has CVID requiring IgRT and immunomodulation. She shares the NFKB1 variant with P1 but is negative for the CXCR4 VUS.

Extensive T and B cell phenotyping revealed low class-switched memory B cell count (0-7 counts/ul) in all subjects, and loss of transitional and mature naïve B cells in P1 and P4 with NFKB1 variant. Proband B cells showed the highest tendency for apoptosis (35-55%) within the family.

Discussion

We describe members of a family with similar presentation (infections, hypogammaglobinemia, warts), however variable combination of NFKB1 and CXCR4 variants, where either genetic defect or their combination could explain the clinical phenotype. Biochemical consequence of our novel CXCR4 variant is pending. As the proband showed the most severe immune phenotype and neutropenia, we hypothesize that CXCR4 has a synergistic effect on NKFB1 loss-of-function. The contribution of CXCR4 VUS of the clinical phenotype of the two other family members is yet to be determined.

Figure 1



(185) Submission ID#812348

Human Phenotype Ontology (HPO) Driven Exploration of Phenotypic Spectrum of A Primary Immunodeficiency Cohort Referred For Research Exome Sequencing

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Abstract/Case Report Text

Background: The yield of diagnosis by exome sequencing for some primary immunodeficiencies (PID) has been less than the typical diagnostic rate for clinical exome analysis (~30-35%). The relatively low diagnostic rates for certain subtypes of the PIDs may be attributed to variable expressivity and/or an incomplete understanding of the genetic basis, among others. Additionally the extent of multiple diagnoses and phenotypyic expansion in PID is not well explored. Cohorts with high-resolution clinical and genetic data are instrumental for exploring these questions. We evaluated the use of human phenotype ontology (HPO)-annotated datasets to systematically address the prevalence of these issues using a cohort of 1000 individuals with PID who participated in research exome sequencing at the NIAID.

Results: We generated a phenotype dataset of 1000 individuals with PIDs by annotating the clinical features of these subjects obtained from electronic health records (EHR) with HPO terms. Exome sequencing of these 1000 individuals identified 313 probands with a pathogenic or likely pathogenic (P/LP) variant in a gene associated with the respective clinical presentation. We identified 118 probands where the same gene harbored a P/LP variant in at least three unrelated individuals. We used the clinical and genetic data of 118 individuals in the following areas:

1) We identified P/LP variants in AIRE, PIK3CD, NLRP3, FAS, CTLA4, GATA2, CYBB, STAT1 and TNFRSF13B in at least ten patients that explained their clinical presentations. This dataset allowed us to characterize variable expressivity of diseases associated with these genes by capturing the variability in the observed HPO terms among probands with P/LP variants in the same gene. Dimensional reduction of clinical features of probands allowed us to cluster patients sharing similar phenotypic profiles. We found clinical presentation of individuals with monoallelic P/LP variants in AIRE were relatively less variable and clustered more compactly compared to that of individuals with GATA2 variants.

2) The extent of multiple diagnoses in PID is not well explored. The benchmark cohort we developed allowed us to identify candidates for multiple diagnosis or phenotype expansion by comparing the phenotype profile of each patient expressed in HPO terms to the HPO terms typically observed for a given PID. For example, we identified gain-of-function pathogenic variant in PIK3CD in a patient that explained the clinical features of the PID observed in this patient. However, the patient also displayed developmental delay, congenital hemiplegia, cerebral palsy and absent speech. These features are not known to be associated with PIK3CD variants, making this individual a candidate for >1 genetic diagnoses.

Conclusions: We developed a benchmark dataset where clinical features of patients were described using HPO terms. This dataset allowed us to quantify variable expressivity for certain PID subtypes and to systematically identify potential candidates for multiple diagnosis or phenotypic expansion.

(186) Submission ID#812353

Two First Cases Of WHIM Syndrome in Ukraine

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Abstract/Case Report Text

Warts, hypogammaglobulinemia, recurrent infections and myelokathexis syndrome is a rare combined immunodeficiency due to autosomal dominant gain-of-function mutations of CXCR4 chemokine receptor. The late diagnosis of WHIM syndrome in two Ukrainian adolescents highlights the diagnostic challenges in this disease.

Patient 1, 12 year-old girl, had recurrent pneumonia since the first year of age; overall she had 7 episodes of pneumonia. She has suffered from chronic bronchitis for last several years. She had recurrent otitis media and chronic pyelonephritis. Neutropenia was revealed when she was 3 year old. During episodes of bacterial infections she occasionally had normal value of neutrophils. The girl does not receive any treatment.

Patient 2, 14 year-old boy, had three episodes of pneumonia when he was 2, 7 and 14 year old. Others symptoms include recurrent herpetic infection, warts on the hands. Since 2 years of age he has haven persistent low neutrophil counts. The child was followed by hematologist and since 5 years of age he has received G-CSF (5 mg/kg) twice a month.

Both children have leukopenia 750 – 1200 cells/mm3, neutropenia – 100 – 300 cells/mm3, lymphopenia 560-830 cells/mm3, low number of B-cells – 30-50 cells/mm3. Hypogammaglobulinemia was not prominent in both children, they have slightly decreased level of IgG (7,1 g/l), normal level of IgM (1,16 – 1,44 g/l), patient 1 has low level of IgA 0,24 g/l. Patient 1 does not have protective level of antibodies to diphtheria and tetanus anatoxin, and anti-Hbs antibodies were absent despite complete immunization.

Bone marrow aspirate revealed hypercellular marrow with granulocytic hyperplasia which was characterized by hypersegmented nuclei and cytoplasmic vacuolization of neutrophils.

On molecular analysis of CXCR4, heterozygous mutation c.1000C>T (p.Arg334*), known as R334X mutation, was detected in both patients, confirming the diagnosis of WHIM syndrome.

Replacement therapy with intravenous immunoglobulin was started in both children together with antibacterial prophylaxis and G-SCF. Vaccination with 4-valent vaccine against HPV infection was recommended for both patients.

WHIM syndrome is very rare immunodeficiency but may be underdiagnosed. The awareness about rare forms primary immunodeficiency is very important in clinical practice for early diagnosis and treatment.

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Practice Survey of the Initial Diagnostic Evaluation of Patients with Suspected Immune-Mediated Cytopenias: A NICER Consortium Study

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Abstract/Case Report Text

Background: Immune-mediated cytopenias, i.e. immune thrombocytopenia (ITP), autoimmune hemolytic anemia (AIHA), autoimmune neutropenia (AIN) and Evans syndrome (ES), are recognized as key clinical features in primary immunodeficiencies and immune dysregulatory syndromes. Upwards of 65% of patients with ES and an uncertain fraction of ITP, AIHA and AIN have pathogenic variants in an immune-related gene. The initial evaluation of patients with immune-mediated cytopenias is not standardized, particularly across sub-specialties. The North American Immuno-Hematology Clinical Education and Research (NICER) Consortium assessed current practices in the evaluation of immune-mediated cytopenias.

Methods: Clinical providers recruited from NICER institutions electively completed web-based survey questions related to provider characteristics as well as initial diagnostic evaluation of ITP, AIHA, AIN and ES via secureQuestionPro® software. Likert scales ranging from 1 ("rarely" < 20%), 2 ("sometimes" 21 to 40%), 3 ("half the time" 41% to 60%), 4 ("frequently" 61 to 80%), and 5 ("almost always" 81 to 100%) were used to ascertain frequency of evaluation for each diagnostic study. Statistical analysis and plotting was done using Rv3.6.1. Plots were created using packages ggplot2, v3.2.0 and ggiraphExtra v0.2.9. Mean Likert scale scores were calculated for each study for each suspected disease and plotted on radar charts.

Results: The survey was completed by 93 providers, including Hematology/Oncology (48.6%), Rheumatology (16.4%), Allergy/Immunology (17.1%) and other sub-specialties (17.9%). A slight majority of physicians (51%) were fellows or within 5 years of graduation; physician extenders and clinical pharmacists were also respondents. The majority (62.4%) of respondents indicated that ≤ 50 new immune-mediated cytopenia patients were seen at their institution annually. The vast majority of respondents (90.4%) reported evaluating ≤ 25 new ES patients per year at their institution with 60% evaluating ≤ 10 cases annually. Collated data from all respondents showed that in all disease states, the primary evaluation was focused on peripheral destruction mechanisms; the majority of patients are only "sometimes" or "rarely" evaluated for bone marrow failure syndromes, connective tissue disease, immunodeficiency and non-malignant lymphoproliferative disorders, but when done were more likely in ES (Figure 1). Evaluations were biased by sub-specialty with higher degrees of connective tissue focus by Rheumatology and immunodeficiencies by Allergy/Immunology (Table 1).

Genetic sequencing was "frequently" or "almost always" sent in 4.5% of ITP, 7.0% of AIHA, 6.2% of AIN and 32.2% of ES patients. Personal or family history of autoimmune/hyperinflammatory disease, malignancy or cytopenias most strongly influenced the decision to send genetic testing. Lack of insurance coverage/negative financial impact on the patient and concerns about the inability to resolve variants of uncertain significance were the biggest barriers for obtaining genetic testing.

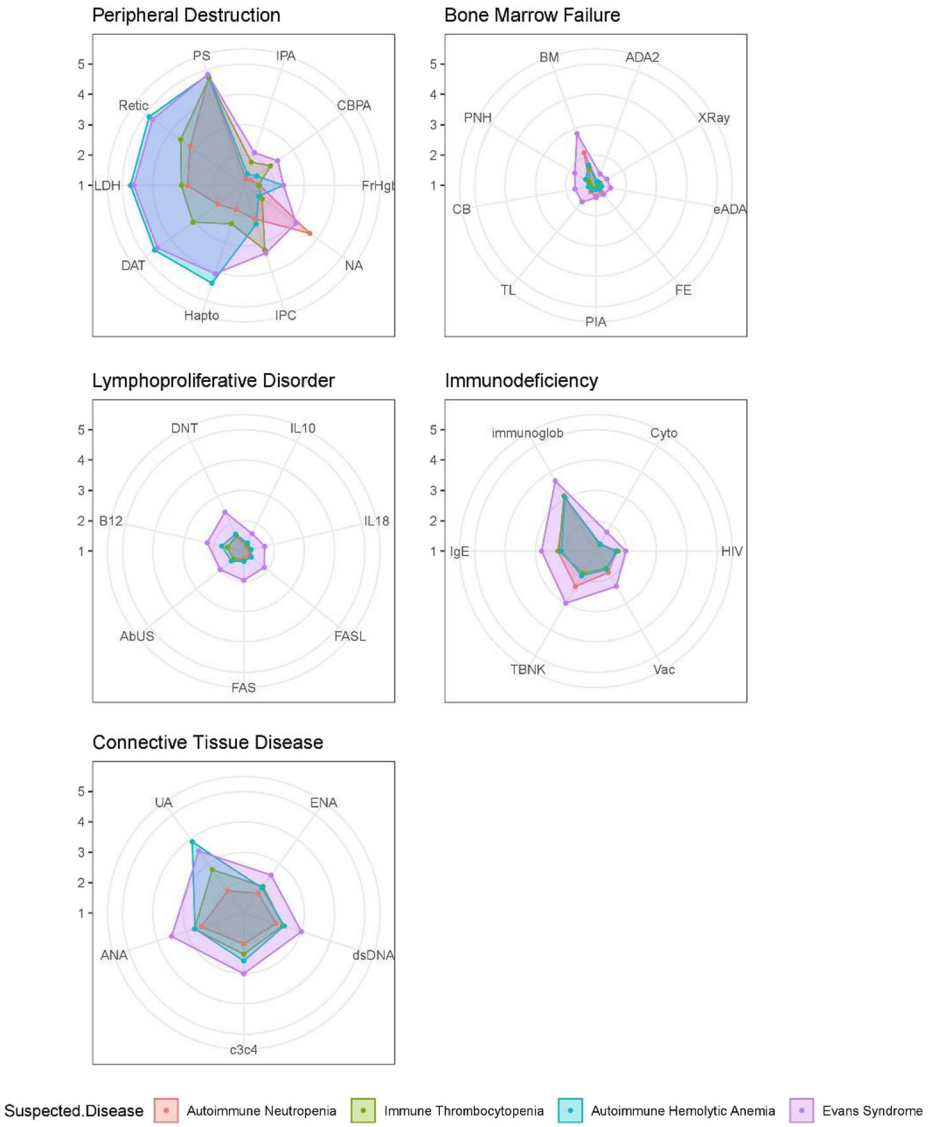
Conclusions: Current practices in the evaluation of immune-cytopenias are heterogeneous by sub-specialty and globally limited in scope with few patients being evaluated for underlying etiologies. In particular, despite a known high frequency of pathogenic variants in ES, less than a third of patients are undergoing sequencing, highlighting a need to reduce barriers to genetic testing. Development of a consensus guideline with multi-disciplinary engagement to harmonize an optimal evaluation for patients with immune-mediated cytopenias is needed.

Table 1. Practice Variation Amongst Subspecialists in Workup of Patients Presenting with Immune Cytopenias

FOCUS OF EVALUATION[#]	SUBSPECIALTY / NUMBER OF RESPONDENTS*				
	Hematology/Oncology (n =53)	Allergy/Immunology (n =8)	Rheumatology (n =14)	Other (n =12)	P value (ANOVA)
Peripheral Destruction	3.06 (0.50)	2.70 (0.68)	2.83 (0.62)	3.33 (0.81)	0.065
Bone Marrow Failure	1.35 (0.38)	1.51 (0.42)	1.11 (0.10)	1.59 (0.49)	0.008
Lymphoproliferative Disorder	1.45 (0.45)	2.55 (1.07)	1.21 (0.30)	1.60 (0.61)	<0.001
Immunodeficiency	2.04 (0.61)	3.12 (0.76)	1.89 (0.56)	2.35 (0.90)	<0.001
Connective Tissue Disease	2.22 (0.71)	2.20 (1.23)	4.20 (0.69)	2.72 (1.09)	<0.001

*87 out of 93 survey responses were evaluable for this analysis

[#]Expressed as average Likert responses on a scale of 1-5 (mean(SD))



(188) Submission ID#812369

IRF2BP2 Deficiency: Expanded Phenotype and Genotype-Phenotype Correlations

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Abstract/Case Report Text

Interferon regulatory factor-2 (IRF2) binding protein-2 (IRF2BP2) was originally identified as a transcriptional co-repressor of IRF2(1). Mutated IRF2BP2 was identified in a 3-member family with recurrent sinopulmonary infections, progressive hypogammaglobulinemia, and poor response to protein vaccines(2). We have now identified 10 additional families (18 subjects) with IRF2BP2 mutations. Clinical histories show an expanded phenotype with 15/21 having chronic gastrointestinal disease; 7 with gastrointestinal manifestations as the initial clinical

complaint. Five had granulomata in liver(x2), spleen, lung(x2) and gastrointestinal tract. Five out of six tested had poor pneumococcal vaccine responses and four patients reported viral infections including Varicella zoster(x2), influenza A and sapovirus.

IRF2BP2 is a 589 amino acid protein containing a highly conserved C-terminal protein-protein interaction Ring domain (RD). Constraint metrics from gnomAD indicate mild tolerance to missense changes and intolerance to loss-of-function alleles. We identified 3 categories of mutations: RD mutation or deletion (n=9 patients), null alleles (n=3) and non-RD missense changes (n=9). Functional studies assessing the ability to affect NFAT-driven luciferase expression were performed. RD mutations (4/5) had more profound loss-of-repression than wild-type, while missense changes had lesser, but still measurable effects. Further, mutation categories and functional studies correlated with clinical phenotypes. Of 9 patients with RD mutations, 8/9 had infections as presenting symptoms, 6/6 tested had hypogammaglobulinemia and 7/9 were diagnosed with CVID. One patient with a missense RD mutation had only an infectious phenotype (pulmonary Mycobacterium avium) with slight decrease in immunoglobulins; in functional studies this mutation had the least effect of the RD mutations. Haploinsufficient patients reported respiratory infections (3/3), recurrent urinary tract infections (2/3), gastrointestinal disease (2/3) and hypogammaglobulinemia (3/3). In contrast, 8/9 patients with non-RD missense changes presented with gastrointestinal complaints while only 2 patients had infections (recurrent bronchitis, shingles). GI disease prevalence is consistent with high levels of IRF2BP2 expression in the colonic crypt cells (Human Protein Atlas). To confirm this, immunohistochemical staining of colon biopsies from two patients was performed, identifying epithelial and glandular cells of the colon.

IRF2BP2 is involved in multiple processes, including the negative regulation of NFAT signaling(3), TCR signaling(4), inflammatory macrophages(5), and PD-L1 transcription(6). Interaction with the glucocorticoid receptor affecting anti-inflammatory and metabolic transcription(7) has also been reported. These observations highlight the IRF2BP2 response to type-I interferons (IRF2) and TCR stimulation (NFAT), regulation of inflammatory macrophages and co-regulation of glucocorticoid receptor mediated signaling. The expanding role of IRF2BP2 in multiple biologic systems correlates with the broad clinical presentation we observed in our patients. Further studies utilizing Irf2bp2 mutation knock-in mice will help characterize the gastrointestinal, lung and immune pathology seen in our cohort.

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(189) Submission ID#812373

A Signaling and Phenotypic Analysis of Common Variable Immunodeficiency Patients through Mass Cytometry

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Abstract/Case Report Text

Common Variable Immunodeficiency (CVID) is a disorder of antibody deficiency arising from over 20 genetic lesions. The clinical presentation of patients with CVID varies from recurrent, severe infections to autoimmunity. The immune dysregulation in CVID is especially difficult to treat and the lifespans of patients suffering from autoimmunity are much shorter than those without such complications. Unfortunately, we have no way to identify which patients fall into which categories, or even know how many sub-categories of CVID there are. Therefore, the field requires a method to classify patients into categories to precisely recognize and aggressively treat the more severe phenotypes. We address this goal by integrating analyses of patient exomes with analyses of cellular signaling. By analyzing stimulation assays with phospho-protein mass cytometry and high-dimensional data analytics, we aimed to elucidate signaling and phenotyping deficiencies in patients with CVID. Importantly, our panel identifies all circulating immune cell subsets in whole blood. In eosinophils, we found amplified responses of pP38, pSTAT3, and Cleaved Caspase-3 in response to TLR1/2 stimulation. We found additional amplified responses of pSTAT3 and pSTAT5 in CD16lo monocytes. This finding suggests a previously unidentified role for eosinophils and CD16lo monocytes to contribute to the pathophysiology of CVID. We found abormal numbers of memory B cell counts, total switched B cell counts, and IgM+, CD38+ B cell (plasmablasts) counts between CVID patients and healthy controls. CD21 expression on B cells was significantly reduced in CVID patients as well. These B cell results mirror findings from prior, seminal studies on CVID. Notably, we have found higher PD-1 expression in the effector CD8 T cells of patients. Integrating phenotype data, genetic analysis, and mass cytometry data will provide a deeper understanding of each patient's phenotype and how the are clustered. We also expect that a better understanding of alterations in the exomes and functions of the circulating immune cells of CVID patients will lead to new therapeutic approaches.

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A Novel Primary Atopic Disorder Associated With A Homozygous Missense Mutation in OSMR

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Abstract/Case Report Text

Objectives: Primary atopic disorders are monogenic disorders leading to profoundly dysregulated allergic responses. Studying patients with these disorders has been instrumental in expanding our understanding of the pathogenesis of allergic inflammation with therapeutic implications for common polygenic versions of allergic disease.

Clinical findings: We have identified a now 8-year old boy who presented with severe eczema, extremely high blood eosinophil counts (5.8x109 cells/L, normal range: 0-0.85x109 cells/L) after birth and very high serum IgE levels (2645υg/L, normal range: 0-500ug/L) since birth. Known allergic disorders and parasitic infections are ruled out. Given the extreme phenotype, whole exome sequencing was performed on the trio of patient and parents, and the patient was found to have a homozygous mutation in the evolutionarily conserved fibronectin III domain of the OSMR gene (c.1307T>A, p.V436D) (Figure 1). OSMR encodes oncostatin M receptor-beta, a component of both the OSM type II receptor and the IL31 receptor, and is important for keratinocyte cell proliferation, differentiation, apoptosis and inflammation. Mutations in OSMR have been reported in association with familial primary localized cutaneous amyloidosis, however this condition was ruled out in this patient through skin biopsy which showed no amyloid deposits.

Methods and results: We modelled the c.1307T>A OSMR mutation in HEK293 cells and observed a loss of expression of the OSMR receptor on the cell surface (with normal intracellular protein levels). This observation was mirrored in primary fibroblasts obtained from the patient. Signal transduction through phosphorylation of STAT1 and STAT5 and gene expression (IL6 and CCL2 measured via qPCR) was absent after stimulation with OSM in patient fibroblasts. These signaling defects were rescued using a lenti-viral transduction approach to introduce the wild-type (WT) OSMR gene. Whole transcriptome analysis using RNA sequencing confirmed that OSM mediated JAK-STAT signalling pathways were deficient in the patient fibroblasts and were rescued after lenti-viral transduction of WT OSMR. RNA sequencing analysis also suggested significantly enhanced expression of genes in the NF-κB signalling pathway (e.g.: IL18 and CXCL1) and decreased expression of genes in the TGF-β signalling pathway (e.g.: Smad6 and Smad7) in patient fibroblasts at baseline. This was also rescued upon lenti-viral transduction.

Conclusion and future directions: Our findings shed light into the disease mechanism of a novel primary atopic disorder, caused by a homozygous missense mutation in OSMR.

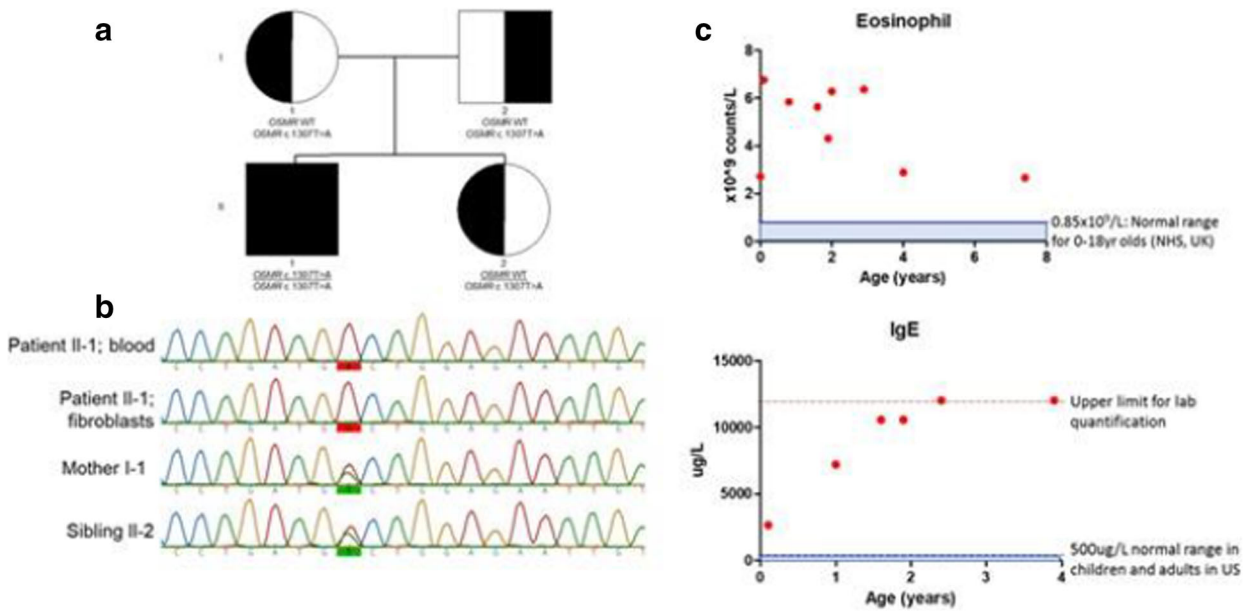

Figure 1. Patient pedigree and clinical findings. a Patient family pedigree and (b) Sequencing of the OSMR c.1307 T>A region in patient blood, patient fibroblasts, mother's blood and sibling blood. c Patient displayed consistently high eosinophil and lgE levels since birth.

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The Other Side Of STIM1: Chronic Myopathy And Platelet Dysfunction In A Patient With A Gain Of Function Mutation In STIM1

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Abstract/Case Report Text

32-year-old Caucasian female presented to Immunology clinic with hypereosinophilia, eosinophilic esophagitis, peptic ulcer disease, severe GI bleeds, and chronic hepatitis. Healthy throughout childhood, with minimal infectious history. In adolescence developed chronic severe myalgias and NSAID overuse, to which the peptic ulcer disease and bleeding were attributed. Parents healthy and non-consanguineous. Son with severe bleeding episodes and small stature. On exam she weighed 82lb, BMI 16. Sclerae anicteric. Tongue deeply furrowed. Cervical nodes palpable. Heart and lung exam normal. No hepatosplenomegaly. No clubbing of the digits or edema. Skin was clear.

WBC 11,600/ul, eosinophils 4730/ul, hemoglobin 10g/dL, normal platelet count. However, platelet aggregation testing abnormal. Bone marrow normocellular, and flow cytometric and molecular analysis did not show hematolymphoid malignancy, primary hypereosinophilic syndrome, or systemic mastocytosis. Lymph node biopsies did not show lymphoma or aberrant T cell populations.

Noted to have chronically elevated creatine phosphokinase, ranging from 706-3164U/L over two years at our institution. Deltoid muscle biopsy showed non-specific myelopathic changes. An adult dystrophy immunostaining panel was normal. Ultrastructure examination showed no abnormal storage material. A genetic panel for metabolic myopathies failed to reveal a cause.

Total IgG, IgA and IgM normal. IgE elevated at 934kU/L, and IgG subclasses showed IgG4 elevated at 354mg/dL. Flow cytometry showed normal T, B and natural killer cell numbers. Normal proportions of naïve, mature and activated T cells. Vaccine response assessment was normal. Evaluation for autoimmune/rheumatologic diseases was negative. Liver biopsy demonstrated findings consistent with primary or secondary sclerosing cholangitis (without increased IgG4 staining).

Given her inflammatory phenotype, additional genetic analysis was sent, assessing for primary immunologic disorders. This identified heterozygous variants of uncertain significance in CTLA4 (c.553T>A; pS185T), ZAP70 (c.981C>G; p.D327E), and STIM1 (c.752T>C; p.L251S). Analysis of the CTLA4 variant in vitro revealed that it was expressed normally. FoxP3 expressing regulatory T cells were present in normal proportions in vivo and appeared phenotypically normal. This variant was found in her unaffected father. The ZAP70 variant is present in population databases (rs201605654, ExAC 0.07%), and was felt unlikely to be clinically relevant.

The STIM1 L251S variant, although not shown previously in human patients, has been previously shown in vitro to be a gain of function mutation[1-3]. Furthermore, familial analysis revealed that this was a de novo mutation arising in the patient, and present in her son. Humans with other gain of function mutations in STIM1 and the ORAI1 channel it activates have overlapping syndromes including Stormorken syndrome, Tubular Aggregate Myopathy and York Platelet syndrome, characterized by chronic myopathy and platelet aggregation defects [4]. The STIM1 L251S mutation is predicted to cause constitutive STIM1 activation and calcium influx and likely provides an explanation for the patient's chronic myopathy and abnormal platelet aggregation. Neither eosinophilic disease, nor cholangitis, have been described previously in STIM1 gain of function-related diseases. It is unclear whether these issues are related to this novel STIM1 mutation, or to other genetic or environmental influences. Treatment of diseases caused by overactive CRAC channels is challenging as no pharmacologic inhibitors are yet clinically available.

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(192) Submission ID#812409

Early Use Of Anti Il-1 In Neonatal-Onset Multisystem Inflammatory Disease (NOMID/CINCA)

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NOMID/CINCA syndrome is one of the periodic syndromes associated with cryopyridines. It is a defect in the innate immune system causing excessive activation of the inflammasome, with consequent IL-1 secretion and neutrophil recruitment. Clinically, damage occurs to organs such as the skin (neutrophilic urticaria), central nervous system (meningitis and deafness) and joint (arthritis). Levy et al. (2015) evaluated a large series of 136 patients and median onset age was 0.8 years, while the median age at diagnosis was 15 years, although the symptoms initiate in the first days of life. Treatment includes corticosteroids, which act by nonspecifically blocking all inflammatory cytokines, or by blocking IL-1 specifically. If early diagnosis and treatment of the disease is not made, natural evolution leads to motor and adaptive disability and death in 20% of cases already in adolescence due to infection, neurological complications or secondary amyloidosis. We report a 10-month-old male child from nonconsanguineous parents who presented shortly after birth, multiple scaling and erythematous lesions throughout the body, evolving with following symptoms: abdominal abscess, hepatitis, meningitis and pioarthritis. Laboratory tests showed elevation of inflammatory tests (ESR, CRP, amyloid protein A) and leukocytosis. The diagnosis was suspected at the nursery where the patient remained hospitalized for 51 days. A personalized multigene panel was requested. It was identified the variant p.Gly309Val,

heterozygous for NLRP3 gene, not described in the literature, confirming the diagnosis of CINCA/NOMID Syndrome. After discharge, it was introduced prednisolone (1,5mg/kg/day) and anti-interleukin-1 (IL-1). After the second dose, skin lesions and joint edema regressed, weight gain, and neuropsychomotor development improved. This case reports a very early diagnosis of NOMID/CINCA Syndrome. It warns neonatologists and pediatricians about the need of precocious recognition of the syndrome, probably improving the prognosis of the patient.

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Mbl And Ficolin 3 as A Factor Influencing the Severity of M. Leprae Disease

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Abstract/Case Report Text

Background: Leprosy affects more than 208,000 people worldwide. Brazil represents the 3rd. country in the world in leprosy frequency and Maranhão State is an hyperendemic region. The city of Imperatriz (MA) stands out as a reference center in the care of these patients. According to few reports, lectin pathway of complement system may play a role in susceptibility to leprosy. Mannose binding lectin (MBL) and ficolins (FCNs) recognize patterns of sugars and acetylated residues (PAMP), respectively, in a wide variety of pathogens, including M. leprae. High levels of ficolins and MBL may act unfavorably promoting the spread of M. leprae. The present study evaluated the role of ficolin 3 and MBL in M.leprae patients and contacts.
Methods: A cross-sectional case-control analytical study was carried out, evaluating clinical and epidemiological data and serum levels of MBL and FCN3 (ELISA) from July 2018 to April 2019. The study was approved by Ethics Committee and Informed Consent forms were signed before sample collection. Data analysis was performed using the SPSS 22.0 for Windows statistics program. Results: We evaluated 169 serum samples (90 patients and 79 healthy family contacts), 54.4% were female, 32% under 15 years old, 78% African-Brazilian, 65% of the families had more than 3 contacts at home. Clinical data showed multibacillary forms in 73.3%; dimorphic (51%) and Virchowian clinical forms (22.2%), up to 03 affected nerves in 26 (64.4%) and more than 5 lesions in 38 (42.2%). It was observed that 32 (35.6%) had a reaction, being type 1 (75%) more predominant. Disability grade 2 was found in 16 patients (17.8%). In children under 15 years, 62.8% were multibacillary, 48.5% dimorphic and 20% undetermined; 11 (31.4%) also had reactions, 90% type 1 reaction and degree of disability 2 in 11.4% of children with the disease. The evaluation of serum FCN3 and MBL levels for the patients (n = 90) and contacts (n = 79) were 363.36ng/ml and 365.81ng/ml, (p = 0.76), and 3035.91ng/ml (P) and 2744.66ng/ml (C) (p = 0.29), respectively. There were lower values of FCN3 in patients with type 1 reaction (sudden and intense inflammatory processes) versus no reaction (337.23 ng/ml vs 372.86 ng/ml) (p = 0.03) and in patients with disability grade 2 (severe sequelae) versus disability grade 1 (319.74 ng/ml vs 343.25 ng/ml) (p = 0.003). Higher FCN3 values was observed in patients with no disability (383.82 ng/ml) (p = 002). MBL concentrations were higher for patients above 15 years in comparison with patients below that age (3482.69 ng/ml vs 2626.04 ng/ml)(p = 0.02)) and correlated with the occurrence of a multibacillary clinical form.
Conclusions: MBL and FCN3 levels were not different in the patients and contacts of M. leprae, nevertheless the presence of severe forms with sequelae (reaction type 1 and disability grade 2) were associated with lower levels of FCN3. In addition, it is possible that lower MBL levels could influence the higher frequency of multibacillary disease below 15 years old.

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Hyper IgE Syndrome with Associated Pica

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Abstract/Case Report Text

Introduction: Hyper IgE Syndrome (HIES) is a primary immunodeficiency characterized by elevated IgE levels. Symptoms can range from severe eczema, recurrent skin infections or pneumonias, and typical dysmorphic facies. There have been wide non-immunologic presentations in patients with HIES, including retained primary teeth, scoliosis, craniosynostosis, arterial aneurysms and joint hyperextensibility. An association between HIES and autoimmune hemolytic anemia (AIHA) has further been described in the literature. However, there have been no reported cases of HIES in association with iron deficiency anemia and concurrent pica. We present a unique case of a patient with a history of eczema, recurrent skin infections and pica found to have HIES and iron deficiency anemia.
Case presentation: A 5-year-old boy with a history of allergic rhinitis presented to the Allergy & Immunology clinic for evaluation of chronic eczema and recurrent skin infections. The patient had a history of multiple hospitalizations requiring intravenous antibiotics for cellulitis and superinfected eczema since he was an infant. Symptoms were refractory to the use of multiple skin barrier ointments and oral antihistamines. His mother further noted that for the past two months prior to initial evaluation, he developed a fixation with eating crayons, baby powder and chewing on drywall.
Physical exam was notable for a dysmorphic face, broad based nose, pale nasal mucosa with ample clear discharge, high-arched palate and lower incisor supernumerary teeth. His skin was characterized by generalized dryness, lichenification and scaly desquamation with boils on extensor surfaces of knees and elbows. Initial screening for HIES via T-helper 17 functional assay was consistent with decreased expression of IL-17. Genetic testing revealed STAT3 S614G missense pathogenic variant consistent with HIES. CBC was also notable for decreased hemoglobin at 9.9 g/L and MCV of 69 fL. Patient was diagnosed with concurrent HIES and pica in the setting of iron deficiency anemia. Iron supplementation was started and patient's pica improved.
Discussion and Conclusion:
Our patient with HIES had a peculiar initial presentation with the classic signs and symptoms of HIES and pica. The diagnosis of HIES can often be delayed due to the wide range of clinical presentations. To our knowledge, the association of HIES with iron deficiency anemia and pica has been underreported in literature. Screening for anemia should be considered when evaluating patients

with HIES in order to rule out comorbid iron deficiency anemia which can be easily treated with iron supplementation.

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(195) Submission ID#812441

Novel, Heterozygous Mutations in BLNK and LRBA Resulting in CVID phenotype with GLILD, Inflammatory and Autoimmune Phenotype

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Abstract/Case Report Text

Introduction: Common variable immunodeficiency is a primary immunodeficiency with variable and diverse phenotypic presentations. The two main phenotypes include a group which primarily exhibits recurrent infections and a group with or without infections and primarily inflammatory and autoimmune complications. The latter, may lead to a delay in diagnosis and is associated with poorer outcomes and higher morbidity and mortality. (1) Another group of patients present with T-cell defects, lung disease, autoimmunity, and infections and may be diagnosed as having CVID but instead can have mutations in LRBA or PI3 kinase. This subset of patients has been referred to as "CVID-like" in the literature. (2)

Case Presentation: Patient is an 11 year old female who initially presented to an outside facility due to 2 days of fatigue, fever, and abdominal pain. Upon presentation, she was found to have massive splenomegaly, hepatomegaly, and an abnormal chest X-ray showing mediastinal lymphadenopathy and pleural effusion. Laboratory results demonstrated pancytopenia, hypogammaglobulinemia, and low B cells, T cells, and NK cells via flow cytometry. She was transferred to our institution for further work up. She did not have any prior history of recurrent infections, asthma/lung disease, or autoimmune conditions. Initial CT of the chest was consistent with granulomatous lymphocytic interstitial lung disease.

Patient was diagnosed with common variable immunodeficiency with granulomatous lymphocytic interstitial lung disease and was treated initially with high dose IVIG, corticosteroid taper, rituximab, and Imuran. She had interval worsening of PFT and lung disease as shown by CT scan.

Genetic panel for CVID and related conditions revealed 2 variants of unknown significance. One heterozygous mutation in BLNK gene (c.616G>A) and one heterozygous mutation in LRBA gene (c.3914G>A). She was started on infliximab with plans to repeat CT scan in 6 months.

Discussion: Mutations in both BLNK and LRBA have been associated with primary immunodeficiency. Mutations in BLNK, which is located on chromosome 10, have been associated with autosomal recessive agammaglobulinemia. Homozygous or compound heterozygous mutations in LRBA on chromosome 4, can lead to LRBA deficiency which encompasses a wide range of clinical presentations including hypogammaglobulinemia, autoimmune disease, inflammatory bowel disease, antibody deficiency, organomegaly, and recurrent infections. (3) Without genetic testing, the clinical presentation can be difficult to distinguish from common variable immunodeficiency. The patient presented has clinical features that can be seen with mutations in both BLNK and LRBA, however she is heterozygous for both mutations. Further analysis, including measurement of LRBA protein expression, is needed to further define her underlying immunodeficiency so appropriate treatment can be administered.

(196) Submission ID#812443

Novel nonsense IKBKG Mutation in an Infant Presenting with Pneumocystis Jiroveci Pneumonia and Disseminated Mycobacterium Szulgai Infection

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Abstract/Case Report Text

A 5 month-old, previously healthy, unvaccinated male presented with one week of diarrhea and cough and was admitted for dehydration and hypoxemia. His mother and sister both had a history of incontinenti pigmenti (IP). On physical exam, he was alert, afebrile, with tachypnea and subcostal retractions. Enterovirus/rhinovirus and parainfluenza 3 were detected, but he became progressively hypoxemic and eventually required intubation and high-frequency oscillatory ventilation. Chest x-ray showed multifocal bilateral airspace opacities. Empiric treatment for PJP with trimethoprim/sulfamethoxazole and glucocorticoids was started. Tracheal aspirate PCR confirmed P. jiroveci. HIV RNA PCR was negative. IVIG was started due to suspicion for primary immunodeficiency. Although his respiratory status gradually improved, he subsequently developed multiple skin lesions. Skin biopsy grew Mycobacterium szulgai. M. szulgai osteomyelitis of the right fibula and the left nasal bone was also detected, indicating hematogenous spread of the infection. He was started on four-drug anti-mycobacterial therapy and interferon-gamma (Actimmune) at doses ranging from 50 μg/m^2 three times weekly to 100 μg/m^2 qod.

Immune work-up revealed T-cell lymphopenia [CD3+/CD4+ 202/μl (1400-5,100/μL) and CD3+/CD8+ 222/μl (600-2,200/μl)] with an abnormally increased proportion of memory CD4 T-cells compared to naïve cells for age. B-cell numbers were normal, and NK cells were decreased [CD56+CD16+/CD3- 17/μl (100-1000/μL)]. NK cell lytic function by K562 lysis was normal, whereas CD107a degranulation was decreased. The serum IgM level was normal [93 mg/dL (31-103 mg/dL) whereas IgA [124 mg/dL (8-83 mg/dL)] and IgG [1020 mg/dL (165-781 mg/dL)] were elevated. Mononuclear cell cytokine response to ligands for TLR2-TLR1, TLR2-TLR6, TLR3, TLR4, and TLR7-TLR8 was normal. DNA

sequencing revealed a novel nonsense mutation in exon 5 of the IKBKG (p.Gln201Ter (Q201X) (CAG>TAG): c.601 C>T, confirming the diagnosis of NEMO deficiency, which was suspected based on the infectious disease presentation and the maternal history of IP. The diagnosis was further supported by signs of ectodermal dysplasia of teeth that appeared starting at 10 months of age. He underwent HSCT using bone marrow from a 10/10 matched unrelated donor after conditioning with ATG, busulfan, fludarabine and rituximab. Actimmune therapy was continued until 10 days prior to transplant. For GVHD prophylaxis, he received tacrolimus and low-dose methotrexate. He achieved full donor chimerism post-transplant and has had no significant GVHD.
Interesting features of this case include the prominence of IP in mother and sister, which is usually due to female heterozygosity for an IKBKG null allele. Such null alleles when inherited by the male fetus are embryonic lethal. Our patient's nonsense mutation would be expected to result in severely impaired IKBKG protein expression and function. However, the fact that he had was born at term and initially was healthy coupled with his preservation of normal TLR function suggests that his IKBKG allele is likely to be a hypomorphic mutation. Studies are in progress using EBV- transformed B-cell lines from the patient to evaluate IKBKG expression and function. Also of interest, our patient was able to tolerate relatively high doses of interferon-gamma therapy without inflammatory side effects or an adverse impact on engraftment or GVHD.

(197) Submission ID#812451

Autosomal Dominant JAK1 Gain-Of-Function Mutation Drives Myelopoiesis and Dysregulated T Helper Responses Leading To Severe Allergic Inflammation That Is Clinically Responsive To Ruxolitinib

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Abstract/Case Report Text
Background: Primary atopic disorders are caused by genetic mutations that skew the immune system towards severe allergic disease. Germline gain-of-function (GOF) mutations in JAK1 are a newly described monogenic cause of severe atopy, with affected patients demonstrating profound eosinophilia and allergic inflammation. Our initial report of this novel condition identified a dramatic clinical response to the combined JAK1/2 inhibitor ruxolitinib. We aimed to determine the long-term clinical response to ruxolitinib in patients carrying a germline JAK1 GOF mutation, and to characterize the effect of enhanced JAK1 signaling on T lymphocyte effector functions and hematopoiesis.
Methods: Clinical outcomes were evaluated in two pediatric patients carrying the c.1901 C>A (p.A634D) GOF mutation in JAK1 after 3.5 years of ruxolitinib treatment. T cell phenotyping was performed using extracellular surface marker and intracellular cytokine staining by flow cytometry, and by gene expression signature profiling of RNA sequencing data. To evaluate the effect of enhanced JAK1 activity on myelopoeisis, we reprogrammed JAK1 GOF patient-derived peripheral blood mononuclear cells into induced pluripotent stem cells (iPSC) and performed directed myeloid differentiation. RNA sequencing was performed on RNA collected during iPSC myeloid differentiation and from whole blood of affected patients before and after ruxolitinib treatment.
Results: Long-term use of ruxolitinib was associated with improved growth, reduced eosinophilia, and control of allergic inflammation without significant infectious complications, however, anemia represented a dose-limiting adverse effect. T cell immunophenotypic analysis revealed severe T helper (TH) cell skewing towards a TH2 phenotype pre-ruxolitinib treatment, in keeping with the allergic clinical manifestations. Analysis of myeloid differentiation revealed an increased myeloid to erythroid ratio in colonies derived from JAK1 GOF iPSCs compared to controls. RNA sequencing analysis of JAK1 GOF human whole blood and iPSCs compared to controls revealed upregulation of cytokine and cytokine receptor genes implicated in allergic inflammation and early eosinophil precursor commitment, including CSF-1 and the interleukin-33 receptor. Reactome pathway analysis of genes upregulated in both JAK1 GOF iPSC and whole blood compared to controls showed enrichment of several pathways including interferon alpha/beta, interleukin-4/-13 and interleukin-33 signaling.
Conclusions: This work demonstrates a critical role for JAK1 in atopic immune dysregulation, specifically driving a TH2 phenotype and eosinophilia. Combined JAK1/2 inhibition can reverse much of the allergic inflammation, with dramatic clinical effects. This has important implications for our understanding of the pathogenesis and potential therapeutic targets for early life allergic immune dysregulation.

(200) Submission ID#812462

Novel heterozygous dominant activating RAC2 variant in four New Zealand patients with combined immunodeficiency

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Abstract/Case Report Text
RAC2 encodes Ras-related C3 botulinum toxin substrate 2, a member of the Rho family of GTPases that are essential for regulating cell signalling and actin cytoskeleton reorganisation. Heterozygous mutations causing dominant activating phenotype were first described in 2019. To date, the majority of reported patients have

had severe combined immunodeficiency (SCID) and/or severe disease in association with their combined immunodeficiency (CID) necessitating haematopoietic stem cell transplantation (HSCT).

We present clinical and laboratory features of 4 New Zealand patients from the same family with a novel heterozygous missense variant in RAC2 [c.62T>G, p.Ile21Ser (I21S)].

The index patient (P1 - age 8 y, M) has a history of infectious gastroenteritis, Staphylococcal aureus conjunctivitis, recurrent otitis media and recurrent Herpes simplex virus (HSV)-1 cutaneous infections. His 2 siblings (P2 – age 9 y, M; P3 - age 6 y, F) and his mother (P4 – age 42 y, F) all have a history of recurrent viral (HSV-1) and bacterial (Staphylococcal aureus, Streptococcal pyogenes) cutaneous infections and/or recurrent sinopulmonary infections that respond to empiric antimicrobial therapy. Affected family members have chronic lymphopenia involving predominantly CD4+ T (range 53-361 x106/L) and B (range 20-181 x106/L) cell compartments. P1, P2 and P3 all have CD4+ naïve (CD45RA+/CD62L+) T cells (range 40-60% of CD4+ T cells) and normal lymphocyte proliferative response to mitogens. P1, P2 and P3 all have reduced IgM but normal IgG and IgA. Antibody responses to protein antigens are preserved. P1 underwent diagnostic laboratory gene panel evaluation (Blueprint Genetics Primary Immunodeficiency Panel, v3) which identified a heterozygous missense variant I21S in RAC2. The variant has not been observed in a large reference population cohort (gnomAD). Sanger sequencing confirmed presence of the variant in the 3 siblings (P1, P2, P3) and their mother (P4). Affected individuals in this family all have neutrophil vacuolation and an abnormal neutrophil granulation pattern. Their neutrophils all had enhanced superoxide production in response to stimulation by fMLP and PMA as compared to healthy controls'. These findings suggest that RAC2 I21S is an activating mutation causing notable abnormalities in neutrophil morphology and NADPH oxidase activation similar to other recently reported mutations.

This novel mutation expands the phenotypic spectrum of RAC2 activating mutations. Clinical management of affected patients needs to be tailored to their phenotype and disease severity.

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(201) Submission ID#812467

Microbiome, metagenomic and metabolomic signatures distinguish patients with enteropathy associated with inherited CTLA4 haploinsufficiency

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Abstract/Case Report Text

Background and aims: Heterozygous mutations in cytotoxic T-lymphocyte antigen-4 (CTLA4) are associated with recurrent infections, lymphoproliferation, autoimmunity and lymphocytic infiltration of target organs. Disease penetrance can be highly variable even among related family members carrying the same CTLA4 mutation. Our evaluation of a subset of the CTLA4 patient cohort followed at the National Institutes of Health (NIH) revealed that 50% of CTLA4 mutation carriers have gastrointestinal (GI) manifestations which include diarrhea and diffuse lymphocytic enteropathy. Our aim was to determine whether the intestinal microbiome, metagenome and metabolome could distinguish patients with CTLA4 haploinsufficiency (CTLA4-H) based on disease severity, and the presence or absence of GI manifestations.

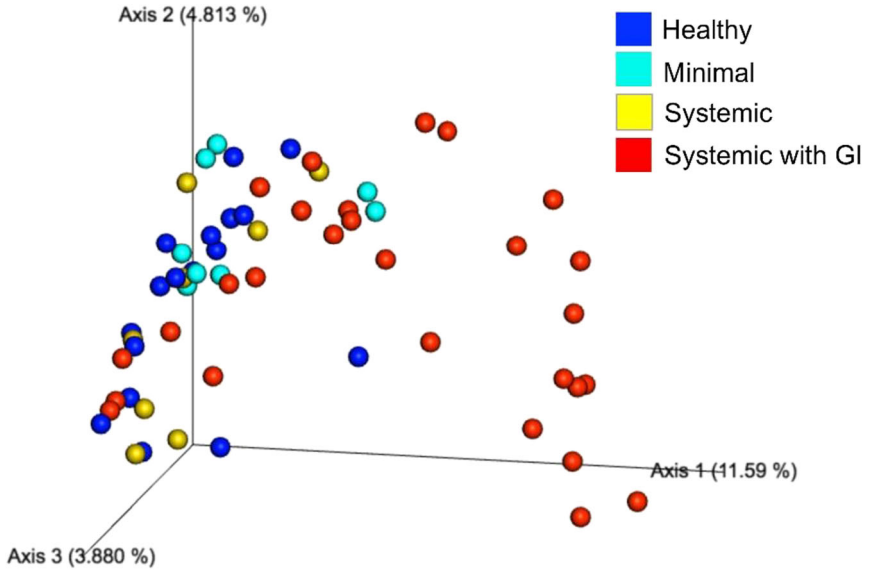
Methods: Clinical metadata and fecal samples were collected from healthy individuals (n=16) and patients with CTLA4-H (n=32). Patients with CTLA4-H were classified as having minimal (n=7, only endocrine and/or dermatological manifestations) or systemic disease (n=25, hematological and multi-organ involvement). They were further classified based on whether they had a history of enteropathy (n=18) or active GI disease (< 2 bowel movements per day and/or blood or mucus in stool) at time of sampling (n=8). Metabolomic profiling (using a panel of 150 metabolites) and 16S rRNA gene sequencing (V4 region) was performed on fecal samples (total samples: 62; number of reads/sample: 18,341 to 226,027; median: 74,857). A subset of 20 samples were subjected to shotgun metagenomic sequencing based on findings from the 16S rRNA gene sequencing analysis.

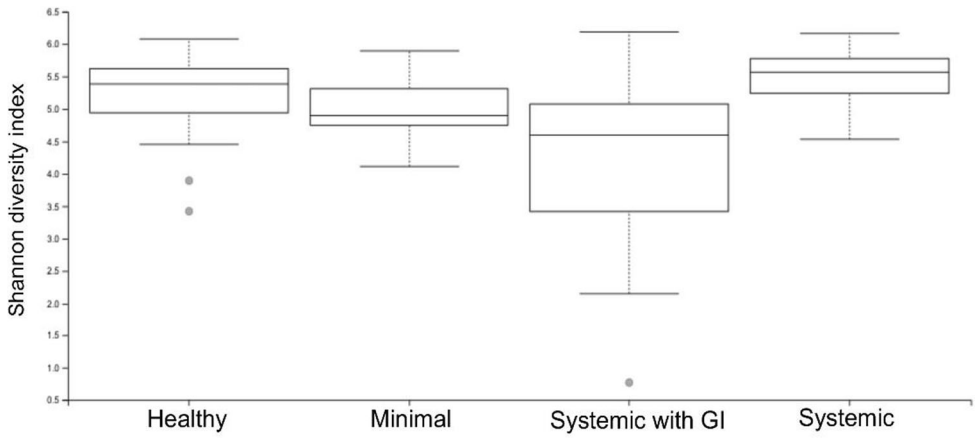
Results: All patients with CTLA4-H and a history of enteropathy or active GI disease also had systemic disease. Fecal samples from patients with a history of enteropathy had a distinct microbial community structure (Fig. 1) which was significantly less diverse (Fig. 2) compared to healthy individuals and patients with minimal vs. systemic CTLA4-H. Patients with a history of enteropathy had significantly higher relative abundance of 5 bacterial taxa including Shigella-Escherichia (Fig. 3). Shotgun metagenomic sequencing confirmed that samples from patients with a history of enteropathy were dominated by subsets of 12 identified Escherichia coli strains, all of which share 30 genes coding for specific types of virulence factors such as curli fibers (facilitate uptake into host cells), flagellar proteins (increase motility) and enterobactins (increase bacterial iron transport). Meanwhile, samples from patients without active GI disease at the time of collection were enriched for several taxa including Bacteroides nordii and Akkermansia muciniphila compared to patients with CTLA4-H and active GI disease (Fig. 4). Metabolomic analyses showed that asparagine, 3-hydroxybutyrate, cytosine and cystine

were enriched in samples with abundant E. coli, whereas samples without E. coli were enriched in metabolites involved in pyrimidine (Holm p=0.0005), purine (Holm p=0.004), and alanine/aspartate/glutamate metabolism (Holm p=0.01) (Fig. 5).

Conclusions: Fecal samples from CTLA4-H patients with a history of enteropathy were heavily colonized with E. coli strains that are associated with a specific metabolomic profile and that share virulence factor genes that may facilitate host invasion. These data suggest that the microbiome and metabolome can distinguish patients with CTLA4-H and GI disease, and support the potential use of antibiotics or even antimetabolites to treat CTLA4-H-related enteropathy.

Figure 1. Beta diversity (Jaccard) of fecal samples from healthy individuals and patients with CTLA4 haploinsufficiency.





Group 1	Group 2	p-value
Healthy (n=18)	Minimal (n=8)	0.34
Healthy (n=18)	SystemicGI (n=28)	0.008
Healthy (n=18)	SystemicNoGI (n=8)	0.29
Minimal (n=8)	SystemicGI (n=28)	0.13
Minimal (n=8)	SystemicNoGI (n=8)	0.12
SystemicGI (n=28)	SystemicNoGI (n=8)	0.008

Figure 2. Alpha diversity of fecal samples from healthy individuals and patients with CTLA4 haploinsufficiency.

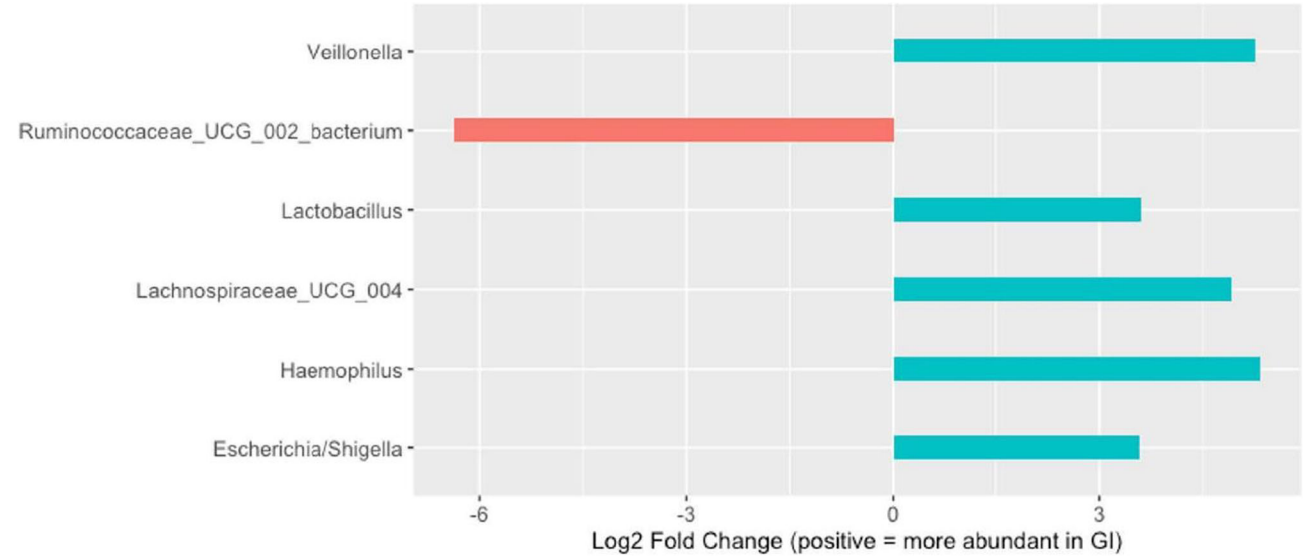


Figure 3. Differentially represented taxa between patients with CTLA4 haploinsufficiency and history of enteropathy vs. no history of enteropathy.

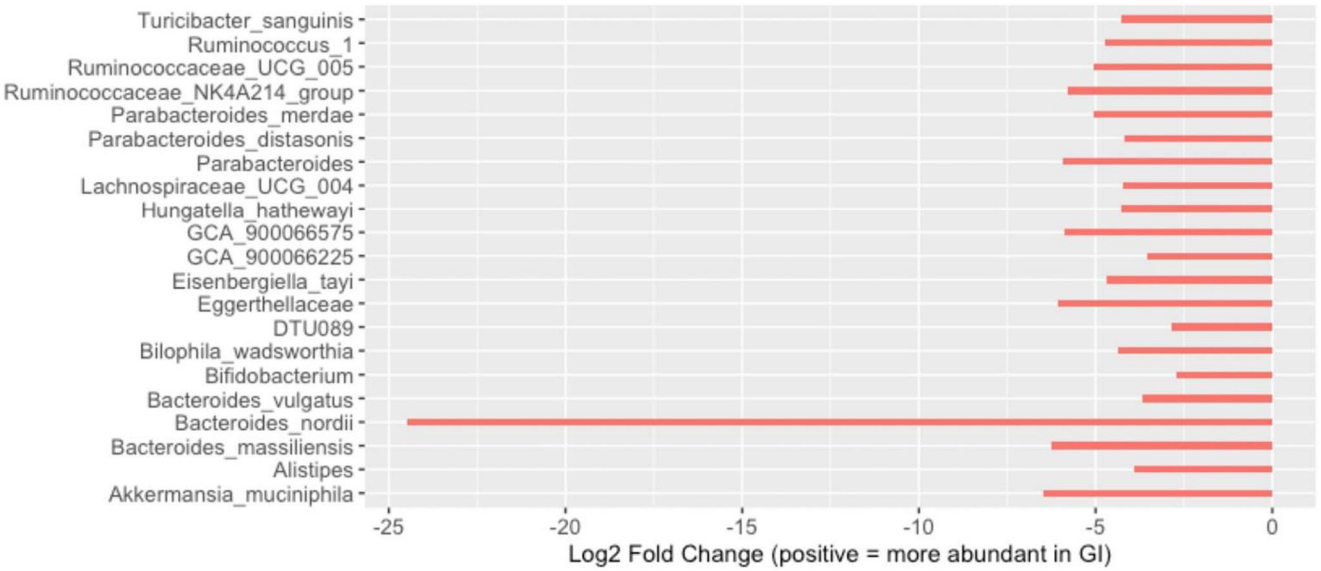


Figure 4. Differentially represented taxa between patients with CTLA4 haploinsufficiency and active GI disease vs. no GI disease at time of sampling.

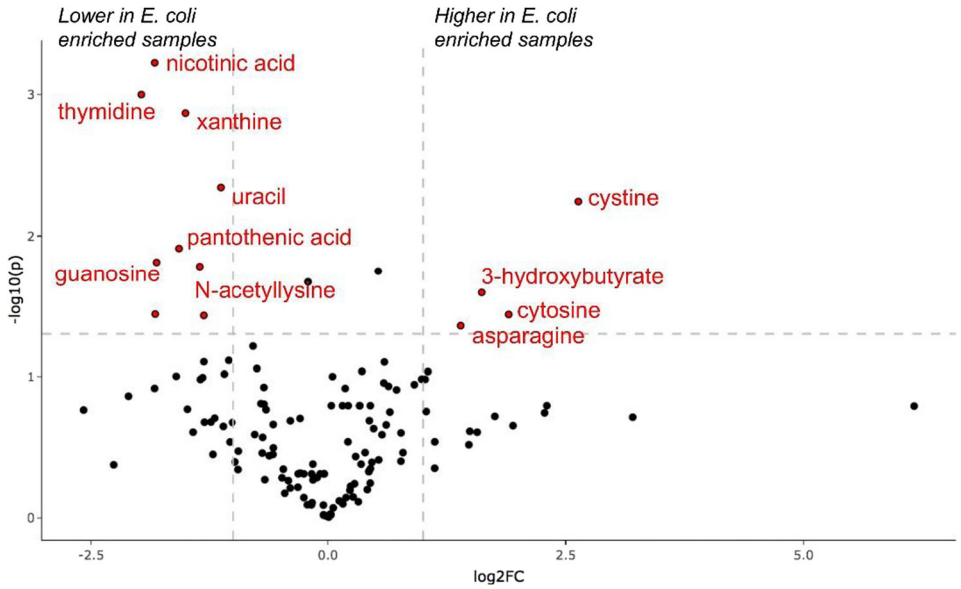


Figure 5. Volcano plot of metabolites present in fecal samples enriched with *E. coli* vs. samples without *E. coli*.

(202) Submission ID#812475

A novel case of DNA polymerase δ 1 (POLD1) deficiency reveals an important role of POLD1 in DNA repair and B cell maturation

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Abstract/Case Report Text
The DNA polymerase delta (Pol δ) complex is essential for leading and lagging DNA strand synthesis. Its catalytic subunit (POLD1), carries both polymerase and exonuclease activities and plays a crucial role in DNA replication and repair. Heterozygous POLD1 mutations have been associated with inherited colorectal cancer and mandibular hypoplasia, deafness, progeroid features and lipodystrophy (MDPL) syndrome. More recently a biallelic loss of function mutation in POLD1 (p.R1060C) that impairs the stability of the POL δ complex, has been reported in 3 related subjects with recurrent infections, deafness and combined immunodeficiency (CID) with T-cell lymphopenia, CD8+ T cell oligoclonality but preserved B cell proliferation.
We report here a second family in which a novel biallelic missense mutation in POLD1 gene was associated with CID. The proband is a 9-year-old boy born to consanguineous Pakistani parents. Since infancy he suffered from failure to thrive and recurrent infections, including 5 episodes of pneumonias, multiple otitis media, sinusitis, recurrent cellulitis at the G tube site, BK viruria and shingles. Live and dead vaccines were well tolerated. At 2 years of age sensorineural hearing loss together with profound leukopenia (ANC 260 cell/μl, ALC 680 cells/μl) and hypogammaglobulinemia (420 mg/dL) were identified. Intermittent IVIG replacement and antimicrobial prophylaxis were initiated.
Immunophenotyping at 9 years of age showed severe T cell lymphopenia (122 CD3+ cells/μl, 68 CD4+ cells/μl, 39 CD8+ cells/μl,

148 CD19+ cells/μl, 25 CD56+CD16+ cell/μl, 9 CD4+CD31+ cells/μl, 13 CD4+CD25hiFoxp3+ cells/μl), and hypogammaglobulinemia (IgM 23 mg/dL, IgG 276 mg/dL, IgA < 10). Physical exam was remarkable for multiple acquired nevi in the groin area, teeth abnormalities and global developmental delay.

Whole exome sequencing analysis revealed a homozygous POLD1 missense variant (NM_001256849 c.3175C>G, p.Q1059E) absent in public databases (CADD score of 24). Parents were heterozygous. TCR-Vβ family expression was normal in both CD4+ and CD8+ T cells, but the proportion of T cells expressing Vα 7.2 (encoded by the distal TRAV1-2 gene) was markedly reduced (less than 1%), consistent with impaired VDJ recombination at the TRA locus and/or with defective thymocyte survival. Constitutive expression of γH2AX was observed in T and NK cells after 1 h and 24h of culture in unirradiated conditions. At 1 h post-irradiation (2 Gy), reduced levels of p-ATM were detected in T and NK cells, and lack of ATM, SMC1 and H2AX phosphorylation was observed in a subset of B cells, suggesting inability of these cells to mount an effective DNA repair response. Bone marrow examination showed normal trilineage hematopoiesis but decreased proportion of CD10-CD20+ mature B cells and increased proportion of pre-B cells.

Conclusion: We report the second mutation associated with autosomal recessive POLD1 deficiency. Our findings broaden the understanding of the mechanisms underlying the immune defect in this disease to include B cell maturation arrest in the bone marrow and a DNA repair defect that may support the generation of a restricted TCR repertoire in the thymus and increased malignancy risk.

Funding: This work was supported by the Division of Intramural Research, NIAID, NIH.

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Low T-cell receptor β (TRB) Repertoire Diversity Early Post-transplant for Severe Combined Immunodeficiency SCID Predicts Subsequent Failure of Immune Reconstitution

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Abstract/Case Report Text

Following allogeneic hematopoietic cell transplantation (HCT) for SCID, the development of a diverse T cell repertoire is essential for optimal immune recovery. High-throughput sequencing (HTS) of the TRB repertoire is the best tool for the evaluation of clonotype dynamics during immune reconstitution as compared to CDR3 spectratyping and staining of Vβ families. We investigated whether longitudinal HTS analysis of TRB would accurately assess development of TCR repertoire diversity over time and reflect the quality of T cell reconstitution following HCT for SCID. We wanted to study the effect of conditioning regimen, SCID genotype, donor type on TCR diversity post HCT. We hypothesized that repertoire diversity may represent an early biomarker to predict long-term immune reconstitution vs. need for a second intervention. We assessed if the TRB repertoire post-HCT carried a molecular signature of self-reactivity.

Methods: The composition and diversity of TRB repertoire of 27 SCID infants, pre-HCT and at 100 d, 6 and 12 mo and yearly post-treatment(s) was studied by HTS. Median time of follow-up was 48 mo. Subjects were part of a prospective study of SCID by the Primary Immunodeficiency Treatment Consortium. Equal amounts of total RNA extracted from peripheral blood was used as template to semi-quantitatively amplify TRB rearrangements. The VDJ statistics file (PAST program) was used to calculate a Shannon entropy (H) index of repertoire diversity and Simpson (1-D) index of repertoire clonality.

Results: TRB sequence analysis of 27 SCID patients showed poor diversity at baseline, followed by improvement to normal complexity (H index >8.0) after HCT. Similar kinetics of development of TRB diversity were seen in patients with IL2RG, JAK3, and IL7R defects (n=16) as in those with RAG and Artemis defects (n=14). In the latter group, however, HCT with no conditioning or immune suppression only was associated with persistently lower diversity than HCT with conditioning ($p < 0.01$), a difference not found in the IL2RG/JAK3/IL7R group (Fig.2). HCT from a matched donor (6/13 conditioned) correlated with higher diversity than HCT from a mismatched donor (5/15 conditioned) (p=0.01). Having > 500 CD4+ T cells/ul at 6 mo post-HCT correlated with higher TRB diversity at 24 and 36 mo post-HCT ($p < 0.01$). The TRB repertoire 100 d post-HCT was enriched for the presence of central cysteines at the apex of the CDR3 ($p < 0.001$), a biomarker of self-reactivity (fig.1). An H-index of 4.7 or lower at 100 d after HCT predicted need for second intervention (HCT or GT)(Fig.3).

Conclusions: Analysis of TRB diversity allows for detailed assessment of development of a diverse T cell repertoire following cellular therapies for SCID and confirms the need for patient-tailored treatment strategies based on SCID genotype. T-cell repertoire 100 d post-HCT is characterized by a molecular signature

that may contribute to the increased rate of autoimmunity early post-transplant. Furthermore analysis of TRB diversity at 100 d post-HCT may identify patients at risk for failure of sustained immune reconstitution, thus prompting a second intervention without delay.

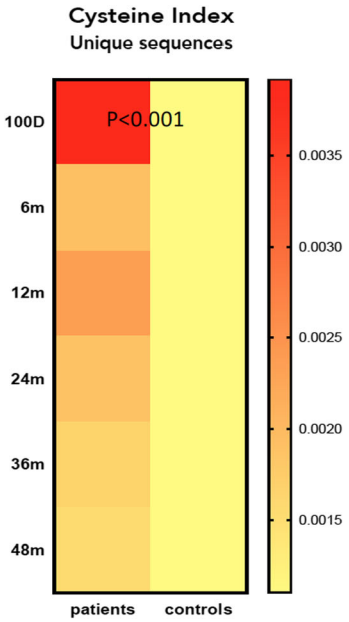

Figure 1. Frequency of TCRβ Unique sequences with a self-reactive CDR3 (Cysteines at the apex) in patients post-HCT and untransplanted controls.

Supported by the Division of Allergy, Immunology and Transplantation, National Institute of Allergy and Infectious Diseases (NIAID); and the Office of Rare Diseases Research (ORDR), National Center for Advancing Translational Sciences (NCATS), National Institutes of Health (NIH), Bethesda, MD, Public Health Service grant/cooperative agreements U54-AI082973 (PIs: J Puck, D Kohn. The Primary Immune Deficiency Treatment Consortium (PIDTC) is a part of the Rare Diseases Clinical Research Network (RDCRN) of ORDR, NCATS.

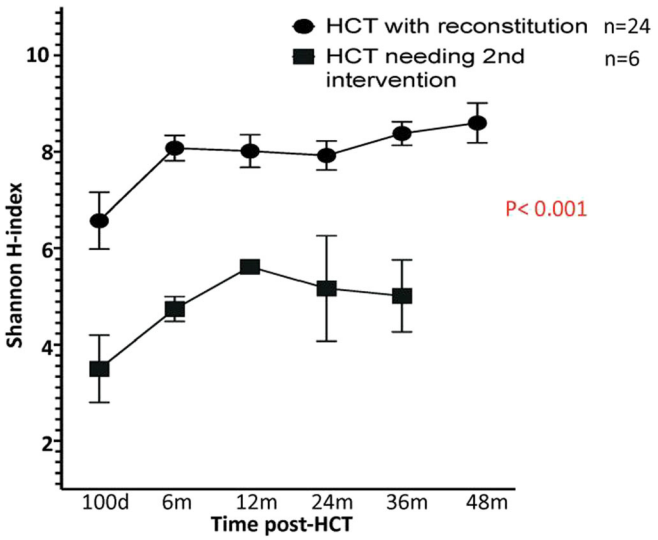

Figure 2. Effect of conditioning regimen on TCR diversity reconstitution based on SCID genotype

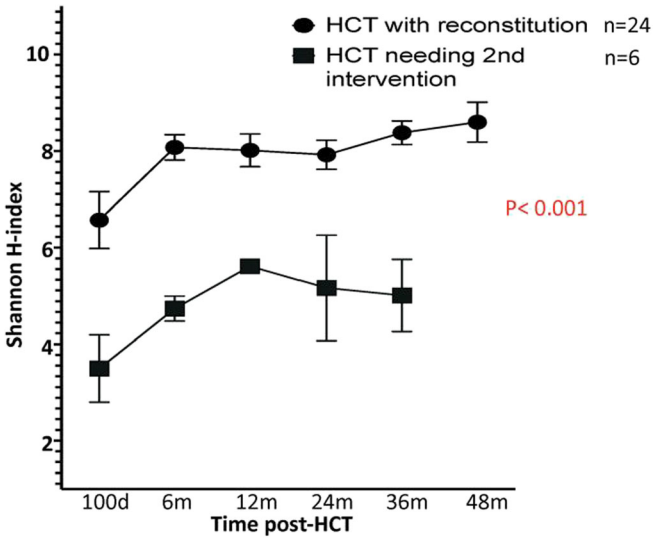

Figure 3. Lower early Shannon diversity score after transplant predicts failed transplant over long term.

(204) Submission ID#812486

The Role Of Skin Biofilm In Pathogenesis Of Atopic Dermatitis

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Abstract/Case Report Text

Background Atopic dermatitis is a chronic, multifactorial, relapsing inflammatory skin condition which is one of the main known health problem worldwide. Atopic dermatitis lesions are frequently colonized by Staphylococcus aureus and Staphylococcus epidermidis. Their susceptibility to form biofilms, ability to form adhesive skin colonies which lead to extremely resistant to antibiotics and immune responses. Formation of skin Biofilm resulted in complex bacterial communities that have unique effects on human keratinocytes, mouse fibroblasts and host immunity.
Aims: The aims of this study to confirm the specificity of S. aureus or its secreted factors in induction of pro-inflammatory cytokines IL-33, TSLP and toxicity on human keratinocytes and mouse fibroblast. The second aim to study the inhibitory effect of co-culture of S. epidermidis with S. aureus in term of production of pro-inflammatory cytokines and toxicity.
Method and materials:
Human epidermal keratinocytes and mouse embryonic fibroblasts cell lines from 3T3 were used as a control strain to examine production of inflammatory response (IL-33 and TSLP) and cell death induced by S. aureus in the presence and absence of S. epidermidis. TSLP and IL-33 were detected by ELISA and the apoptosis of S. aureus and S. epidermidis on these cells was evaluated by flow cytometry.
Result: Recent findings propose the important role of skin biofilms in the pathogenesis of atopic dermatitis. S. aureus have been found to induce secretion of pro-inflammatory cytokines and cause apoptosis of human keratinocytes and mouse fibroblasts. Presence of S. epidermidis as skin biofilm found to protects the human keratinocytes and mouse fibroblasts from induction of pro-inflammatory cytokines and cytotoxicity.
Conclusions and future work: S. aureus are essential in production of inflammatory response and cell death of mouse fibroblasts and human keratinocytes. Future work will be carried out to identify the soluble factors that responsible in induction of pro-inflammatory cytokines. In addition, more studies are needed to be able to understand the mechanism by how S. epidermidis reduce the induction and cytotoxicity caused by S. aureus.

(205) Submission ID#812492

Evaluation of the effect of an Allergy/Immunology (A/I) Training Program on the diagnosis of antibody deficiencies and the Criteria for the use of Immunoglobulin Replacement Therapy (IgRT)

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Abstract/Case Report Text

OBJECTIVES: Immune globulin replacement therapy (IgRT) is indicated for the treatment of antibody deficiencies. The decision to initiate IgRT

in adult patients, in whom arbitrarily defined diagnostic criteria for antibody deficiency syndromes are not fulfilled, is subject to interpretation and decision differences reported by immunologists world-wide. In this study, we explored whether training in one particular program would decrease the variability in diagnostic and treatment approaches seen in the responses to two nationwide questionnaires in the UK and the USA.
METHODS: A 10-minute online survey originally administered to a cross-sectional sample of 203 US allergists/immunologists (USA/I) in January, 2019, was also answered by 43 A/I subspecialists who had trained in the last 25 years at the Louisiana State University Health Science Center Allergy Immunology training program in New Orleans (LAA/I). Respondents were asked questions on patient assessment, antibiotic use, initial IGRT, and immune response assessment in decision-making to prescribe IgRT. USA/I participants were recruited from the Dynata physician professional panel. LAA/I participants were recruited by the Louisiana Primary Immunodeficiency Network (LAPIN).
RESULTS: Overall, LAA/I had consensus responses to the various practice questions close to 90% of the time, but outliers were always present, as was also observed in the USA/I. There was a higher frequency in the reported care of patients as described in the questionnaire by LAA/I.
Over 98% of LAA/I assessed vaccine responses prior to commencing IgG replacement vs only 90% of USA/I $p < 0.5$. All LA A/I used the pneumococcal vaccine for assessment purposes while few used tetanus and Hemophilus influenza, and none used meningitis or salmonella vaccines. These vaccines were still used by some of USA/I. A high level of concordance was observed among all respondents in that only few regarded pneumococcal antibody testing as the definitive test to commence IgRT. High resolution chest CT scan was used more often by LAA/I before starting IgRT. Assessment of effectiveness of IgRT was decided after only 3 months by more USA/I, vs LAA/I, who tended to wait 6 months to decide to continue or discontinue IgRT.
CONCLUSIONS: All A/I responders saw a significant number of patients who do not conform to strict diagnostic criteria for antibody deficiency syndromes. There is diversity in the approach of USA allergists/immunologists in determining the indication for IgRT for non-classical antibody deficient patients. LAA/I responses made It obvious that post graduate influences always play a role in shaping the way A/I practice evolves after graduation.
Drawing on clinician experiences through questionnaires offers a valid contribution to developing consent approaches to improve patients' clinical conditions. Diagnostic criteria and treatment guidelines would benefit from practice-based realistic recommendations based on A/I experience.

(206) Submission ID#812494

A PU.1 Reporter Cell Line Models the Transcriptional Impacts Of Human SPI1 Mutations

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Abstract/Case Report Text
Background: PU.1, a member of the ets transcription factor family, is important in cell fate specification of multipotent hematopoietic progenitors and is encoded by SPI1. Functionally important domains of PU.1 include its C-terminal Ets DNA-binding domain and protein-protein interaction-regulating PEST domain. PU.1 exerts its transcriptional regulation by binding to the purine-rich 5'-GGAA/T-3' λβ DNA consensus sequence (λβ DNA) of hematopoietic lineage-specific enhancer sites via its Ets domain. Pathogenic somatic SPI1 mutations are common in myeloid leukemias and classical Hodgkin lymphoma. We recently identified several SPI1 variants in patients with novel disease we call PU.1-mutated agammaglobulinemia (PU.MA).
Objective: To assess the impacts of SPI1 mutants on transcription we sought to developed PU.1 reporter cell lines.
Methods: To determine if a PU.1 variant proteins could bind-λβ DNA and drive transcription we transfected HEK293 cells, which lack native PU.1, with two vectors, one contained mutated IRFP-2A-SPI1 constitutively driven by the CMV promotor and the other contained EGFP which was placed under control of PU.1-specific λβ promoter/enhancer DNA sequence. Patient SPI1 variants were introduced via site-directed mutagenesis. To determine if mutant PU.1 interferes with the transcriptional activitiy of wild-type PU.1, we created a second PU.1 reporter line transfected with mutated IRFP-2A-SPI1, λβ-EGFP and unmutated mCHERRY-2A-SPI1.
SPI1 transfection efficiency and EGFP expression were evaluated by flow cytometry. Stability of PU.1 expressed in lines was confirmed with immunoblots.
Results: We introduced 5 SPI1 mutations identified in immune deficient patients into IRFP-2A-SPI1. The frequency of EGFP expressing cells from G109Sfs* (6.07%), Y122x (2.04%) H212P (6.68%), V242G (6.54%), and K246del (10.6%) expressing lines were significantly decreased compared to WT PU.1 (29.2%) ($p < 0.001$). Mean geometric fluorescence values for mutated lines were also similarly diminished ($p < 0.05$).
The frequency of EGFP expressing cells transfected with unmutated mCHERRY-2A-SPI1 was not significantly altered by co-transfection with mutated IRFP-2A-SPI1.
Western blots of mutant line lysates revealed that PEST-domain PU.1 mutants (G109Sfs*78 and Y122x) were expressed at very minimal levels while Ets-domain PU.1 mutants were expressed at similar levels as WT PU.1.
Conclusion: Taken together, these data suggest PU.MA SPI1 mutations are damaging and encode either destabilized truncated proteins or transcriptionally inert full-length PU.1 with ETS domain amino substitutions. Hence, PU.MA PU.1 mutants exert their effects through haploinsufficiency.

(207) Submission ID#812498

A Case of Lymphoproliferation with ALPS-like Phenotype

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Abstract/Case Report Text

Background: Autoimmune lymphoproliferative syndrome (ALPS) is a rare genetic disorder secondary to a defective FAS-mediated apoptotic pathway of mature lymphocytes. It is characterized by chronic non-malignant lymphoproliferation in the form of lymphadenopathy and/or splenomegaly, autoimmune manifestations such as cytopenias, increased risk of lymphoma, and expansion of TCRαβ+ CD4-/CD8- (DNT)T-cells. Germline or somatic pathogenic variants in FAS, FASL, and CASP10 are well described genetic defects associated with ALPS. The definitive diagnosis for ALPS, based on the revised 2009 NIH diagnostic criteria, include both required criteria (chronic non-malignant, non-infectious lymphadenopathy, splenomegaly, or both and elevated TCRαβ+ DNT T-cells) and one of the primary accessory criteria (defective lymphocyte apoptosis or mutation in the genes mentioned above). Patients who do not meet the current diagnostic criteria are considered for ALPS-related disorders.

Case presentation:

We report a 2-year-old male who presented with recurrent infections, splenomegaly and chronic lymphadenopathy since 8 month of age. Due to its chronicity he was evaluated by multiple specialists for malignant and infectious causes. Hematological workup including bone marrow biopsy was unremarkable except for an elevated LDH level. Infectious workup identified a past CMV infection. Clinical course is pertinent for chronic splenomegaly which was identified incidentally at 1.5 years of age during an evaluation for intussusception.

Family history is pertinent for a father with recurrent infections, paternal grandmother with thrombocytopenia of unknown cause requiring platelet transfusions, and paternal cousin with neutropenia. There is no family history of lymphomas.

History of chronic lymphoproliferation and recurrent infections prompted an evaluation for lymphoproliferative disorder. Full immune workup was notable for elevated plasma IL-10 and IL-18, normal immunoglobulin levels, lymphocytes subsets, vitamin B12 level, soluble FASL, and relative frequency (%) but borderline increased absolute count of TCRαβ+ (DN) T-cells. In addition, he was noted to have presence of anti-platelet antibodies, poor lymphocyte proliferation to antigens, and low pneumococcal antibody titers. Genetic testing with a 207 PID gene panel identified a likely pathogenic heterozygous variant in PRF1 c.487del (p.His163Thrfs*96), a heterozygous variants of uncertain significance in CASP10 c.683C>T (p.Pro228Leu) and STIM1 c.304A>G (p.Thr102Ala). The CASP10 variant is present in 63 alleles in gnomAD (282K total allele count) and reported deleterious by SIFT.

Discussion: Unlike the typical ALPS presentation, characterized by dominantly lymphoproliferation and autoimmunity, our patient's clinical phenotype is striking for recurrent infections, abnormal T-cell function, and poor antibody response. Our patient does not the meet diagnostic criteria for ALPS due to normal relative frequency of DN T-cells. However, presence of elevated of IL-10, IL-18, platelet autoantibodies raise concern for ALPS-related disorder. In addition, family history of recurrent infections and cytopenias raises concern for familial autoimmunity and ALPS-like phenotype. Although CASP10 is associated with autosomal dominant and autosomal recessive ALPS, the role of this VUS is yet to be determined.

Conclusion: We continue to investigate the pathogenicity of our novel CASP10 VUS. Further studies include pedigree analysis, Fas apoptosis assay and apoptosis pathway testing to assess for the etiology of this ALPS-related disorder. (word count 487, max 500)

Laboratory studies:

Absolute αβ TCR+ DNT	61 cells/ μL	37 cell/uL
Absolute αβ TCR+ DNT B220+	19 cells/ μL	0.0
% αβ TCR+ DNT	2%	0.9%
% αβ TCR+ DNT B220+	0.6%	0.0
Soluble Fas-Ligand	439 pg/mL (69-493)	
IL-10	11.8 pg/mL (<2)	
IL-18	848 pg/mL (89-540)	
Vitamin B12	721 pg/mL (180-914)	

(208) Submission ID#812503

Retrospective Analysis of the Immunologic Evaluation in Patients Receiving Ocrelizumab

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Abstract/Case Report Text

Rationale: Ocrelizumab is a recombinant anti-CD20 monoclonal antibody, which binds to a different, but overlapping CD20 epitope than rituximab. There have been increasing reports evaluating hypogammaglobulinemia and morbidity and mortality in patients receiving rituximab, but there is a paucity of data on hypogammaglobulinemia in patients treated with ocrelizumab.

Methods: We performed a retrospective review of patients who received ocrelizumab in our healthcare system. We evaluated the demographics, indication for ocrelizumab, frequency of immunologic evaluation, and hypogammaglobulinemia pre- and post-ocrelizumab. Hypogammaglobulinemia was stratified as mild (IgG < 600mg/dL or less than lab reference range), moderate (IgG < 400mg/dL) or severe (IgG < 200mg/dL).

Results: We identified 185 patients who received ocrelizumab for multiple sclerosis (average number of ocrelizumab cycles = 4; range 1-9 cycles). There were 120 (65%) female patients, with a mean age of 49 years old (range 23-74; standard deviation ±13). 135/185 (73%) patients had their immunoglobulins evaluated at any time prior to ocrelizumab. 154/185 (83%) patients had their immunoglobulins evaluated at any time following ocrelizumab. Of these, 27/135 (20%) patients had hypogammaglobulinemia (low IgG) pre-ocrelizumab. The majority of these patients had mild hypogammaglobulinemia (25/27 [93%]), with only 1/27 (4%) patients having moderate hypogammaglobulinemia, and 1/27 (4%) patients having severe hypogammaglobulinemia pre-ocrelizumab. Following ocrelizumab, 35/154 (23%) patients had hypogammaglobulinemia, with again the majority of patients having mild hypogammaglobulinemia (34/35 patients [97%]), and 1/35 (3%) patient had moderate hypogammaglobulinemia. In the 116 patients who had both pre- and post- ocrelizumab immunoglobulins evaluated, immunoglobulin levels decreased post-ocrelizumab (p=0.0008).

Conclusions: Approximately 1 in 5 patients receiving ocrelizumab have hypogammaglobulinemia either pre- and/or post-ocrelizumab. There was a statistically significant decrease in immunoglobulin levels post-ocrelizumab. Future studies are needed to evaluate infectious outcomes and complications to see if the hypogammaglobulinemia is associated with inferior outcomes with excess morbidity and mortality.

(209) Submission ID#812509

DOCK8 Deficiency Presenting As Chronic, Diffuse, And Recalcitrant Molluscum Contagiosum

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Abstract/Case Report Text

Introduction: Dedicator of cytokinesis (DOCK) 8 deficiency is an autosomal recessive hyper-IgE syndrome that typically presents as severe atopy, recurrent Staphylococcus aureus abscesses, pneumonia, viral cutaneous infections, and malignancies. Here we present a case of DOCK8 deficiency presenting as chronic, recalcitrant molluscum contagiosum.

Case Description: A 6 y.o. female with severe eczema and a 12-month history of severe molluscum contagiosum was referred to our office. Of note, she was initially seen in immunology clinic at age 2 due to an episode of pneumonia requiring hospitalization and immune evaluation demonstrated mild hypogammaglobulinemia, elevated IgE (8035) and hypereosinophilia. She was subsequently lost to follow up. Three years later, she was referred by dermatology to immunology for diffuse and recalcitrant molluscum contagiosum in the context of challenging to manage eczema, having failed multiple therapies for molluscum including imiquimod, Zymaderm, cimetidine, and curettage of roughly 100 lesions under sedation. She had also had frequent bacterial superinfection of her atopic dermatitis. She was fully immunized and live vaccines were well tolerated. Her family history was negative for primary immune deficiency and consanguinity, with multiple healthy siblings. Her presenting physical exam was notable for widespread eczematous patches and thinly lichenified plaques over her face, chest, arms, back and legs with innumerable, variably sized (many large), juicy, pearly, pink and skin colored, umbilicated papules. The constellation of severe and exuberant cutaneous viral infection, eczema, food allergies, elevated IgE and hypereosinophilia and hypogammaglobulinemia raised concern for combined immunodeficiency. Her immune profile demonstrated low CD8T cells, low switched memory B cells, low IgM, reduced vaccine titers, impaired T cell function by mitogen stimulation and normal count of Th17 cells. A whole exome sequence revealed compound heterozygous mutations in DOCK8, including a large deletion and a frameshift mutation in DOCK8 with absent DOCK8 protein by flow cytometry. Bacterial and fungal prophylaxis and subcutaneous Ig replacement was started. A decision was made with the family to pursue hematopoetic stem cell transplant (HSCT). Her sibling was a 10/10 match and she underwent matched sibling donor HSCT conditioned with busulfan, fludarabine and anti-thymocyte globulin. She is currently 18 months post-transplant and doing well with 100% engraftment, no symptoms of GVHD, and she is off immune suppression with normal T, NK and B cell counts, as well as normal IgG, IgA and IgM levels. Her eczema and Molluscum entirely resolved post-HSCT.

Conclusion: In patients presenting with severe, recalcitrant molluscum contagiosum in the setting of severe atopic disease it is important to consider primary immunodeficiency in the differential and genetic testing should be part of the initial evaluation.

(210) Submission ID#812510

Early Onset Autoimmune Enteropathy In An Infant With CTLA4 Haploinsufficiency

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Abstract/Case Report Text

Rationale: CTLA4 is an inhibitory receptor of T-cell proliferation, integral to immune homeostasis and tolerance. Germline heterozygous loss-of-function mutations result in immune dysregulation, immunodeficiency, and lymphoproliferation. Aggregated data from a cohort of 133 patients found the median symptom onset age was 11 years (range < 1–59 years); only one patient presented under 1 year old at 3 months with recurrent respiratory and gastrointestinal infections , .

Methods: Retrospective chart review, including immunology, pathology, and genetic analysis.

Results: A full-term 6-month-old boy with normal TREC screen and maternal familial history of CTLA4 haploinsufficiency was hospitalized for failure to thrive (height and weight < 2%) and refractory diarrhea starting at 4 months. Stool calprotectin 515mg/kg (normalT (p.R51). Endoscopies demonstrated flattened duodenal villi with biopsies yielding CD3-predominant lymphocytic infiltrates throughout. Expression of Treg FOXP3 was significantly decreased and CTLA4 expression was also decreased by flow cytometry.

Diarrhea and weight gain initially improved with continuous elemental formula by nasogastric tube. At 7 months of age, patient developed Clostridium difficile colitis with clearance of toxin PCR with a course of metronidazole. A gastronomy tube was placed at eight months of age, but weight gain remained poor. Although infectious surveillance remained negative, patient developed hypergammaglobulinemia with IgG level of 1120 mg/dL with serological autoimmune screens pending. Repeat endoscopy yielded unchanged findings at 10 months and it is planned to initiate CTLA4-Ig (abatacept) at 10 mg/kg.

Conclusions: CTLA4 haploinsufficiency exhibits a wide variance in phenotypes and age of onset. We present one of the earliest published patients who developed autoimmunity, requiring early initiation of CTLA4-Ig. Indications for abatacept therapy as well as the timing of bone marrow transplant have not been established in very young patients. Further investigation needed to establish clinical protocols in this age group as well as the etiology of the variability in disease penetrance and expression.

(211) Submission ID#812513

Inflammatory Cytokine Expression in CD4+ T-cells of Patients with Chronic Granulomatous Disease

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Abstract/Case Report Text

Background: Chronic granulomatous disease (CGD), due to defects in NADPH oxidase subunits, is associated with increased susceptibility to severe bacterial and fungal infections. Along with infections, patients with CGD also suffer from a wide range of inflammatory conditions including inflammatory bowel disease, inflammatory respiratory disease, and systemic autoimmune disease. Previous studies have demonstrated increased inflammatory cytokine production in macrophages, monocytes, neutrophils, and CD4+ T-cells of patients with CGD. The precise mechanisms for inflammatory disease in CGD and how they correlate with clinical symptoms are not known. Additionally, there are no established biomarkers that correlate with inflammatory manifestations or control of disease. We aimed to determine if increased inflammatory cytokines correlates with clinical symptoms.
Methods: Peripheral blood mononuclear cell (PBMC) aliquots were collected from 12 CGD patients with varied clinical statuses and 5 healthy donors. Samples were prepped, incubated, stimulated, stained, and fixed before being analyzed via flow cytometry to determine proportions of the intracellular cytokines IL-6, IL-10, IL-17A, IFNγ, and TNFα relative to the total CD4+ population per sample. Nine patients had single time points of intracellular cytokine activity and 3 patients had multiple time points analyzed. Samples were analyzed and compared to the healthy control group and across clinical status.
Results: Of the 12 CGD patients, 4 had X-linked and 8 autosomal recessive CGD (6 p47phox and 2 p22phox mutations). Four CGD patients had active colitis, 3 were being treated with immunosuppression, including corticosteroids, at the time of PBMC collection. Patients with CGD demonstrated statistically significant elevations in the percentage of intracellular IL-6 (0.37% vs. 0.26%, p=0.035) and IL-17A (1.24% vs. 0.71%, p=0.0001) in stimulated CD4+ T-cells. TNFα, IFNγ, and IL-10 were not statistically different between CGD patients and healthy controls. TNFα, IFNy, IL-10, and IL-17A expression in patients with CGD who had active colitis or history of colitis were increased as compared to CGD patients without a history of colitis but did not reach statistical significance. In two patients, IL-17A expression that was elevated pre-HCT normalized post-HCT.
Discussion: The mechanism for increased susceptibility to inflammatory disorders in patients with CGD has not been well elucidated. Our results agree with previous studies demonstrating increased IL-6 and IL-17A production from CD4+ T-cells in patients with CGD indicating a pro-inflammatory state in these patients at baseline. Also, there appears to be an increase in TNFα, IFNy, IL-10, and IL-17A expression from CD4+ T-cells that correlates with presence of inflammatory disease vs. those without inflammatory disease indicating that these cytokine perturbations may be able to serve as biomarkers of disease activity. A larger sample size with prospective collection will be analyzed in the future.

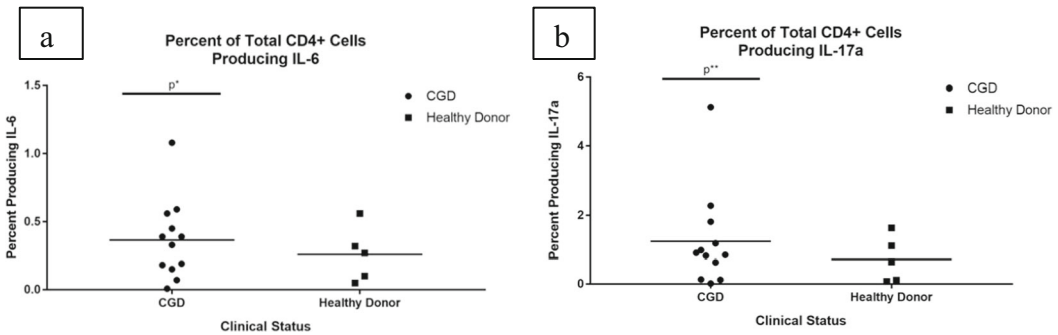

Figure 1. Percentage of cytokine producing CD4+ T-cells in patients with chronic granulomatous disease (CGD) compared to healthy donors. A. Percentage of IL-6 positive CD4+ T-cells, *p=0.035. B. Percentage of IL-17A positive CD4+ T-cells, **p=0.0001.

(212) Submission ID#812521

A Case of G6PC3 Congenital Neutropenia, Misdiagnosed As Evans Syndrome

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Abstract/Case Report Text

Background: Glucose-6-phosphatase catalytic subunit 3 (G6PC3) deficiency, is characterized by severe congenital neutropenia, recurrent bacterial infections, mild intermittent thrombocytopenia and a high incidence of congenital cardiac and uro-genital defects. We report the case of a 28-yo male with chronic neutropenia and thrombocytopenia, who was found to have homozygous pathogenic variants in G6PC3 (c.210del, p.Phe71Serfs*46). Unique to this case is the patient's long history of misdiagnosis of Evans syndrome (chronic autoimmune neutropenia with thrombocytopenia).
Case presentation: A 28-yo male with reported diagnosis of chronic autoimmune neutropenia and thrombocytopenia since age 7 was referred to our

clinic with concern for an underlying immune dysregulation syndrome. He had a history of oral ulcers, gingivitis, recurrent bacterial infections (otitis media, pneumonia, skin abscess) concomitant with severe neutropenia (< 500 cells/uL), for which he had received treatment with systemic steroids and G-CSF since he was 9 years old. He also had history of asthma and short stature, thought to be secondary to his chronic systemic steroid use. His physical exam was otherwise only notable for mild gingivitis.

Workup: Previous diagnostic studies were negative for Fanconi anemia (by DEB testing) and mutations in ELANE. Multiple bone marrow aspirates/biopsies showed normal trilineage hematopoiesis and were otherwise unrevealing. A targeted next-generation-sequencing panel to assess for actionable mutations in genes recurrently altered in myeloid and lymphoid neoplasms (heme-STAMP) revealed a likely pathogenic KRAS mutation at 2% variant allele frequency in peripheral blood. A comprehensive immune work up in our clinic revealed normal B, T and NK subsets. IgG level was increased at 1980 mg/dl. He had protective titers to diphtheria, tetanus, varicella and mounted 15/23 protective titers for pneumococcal (65%). Neutrophil autoantibodies were negative. A repeat bone marrow biopsy was now hypercellular secondary to a marked hyperplasia with increased myeloid precursors as well as an accumulation of mature neutrophils, a subset of which showed pyknotic nuclear alterations. However, despite the myeloid hyperplasia, the patient was neutropenic. These findings are consistent with myelokathexis, abnormal mature neutrophil retention in the bone marrow. Work up for genetic causes of immunodeficiency immune dysregulation revealed homozygous pathogenic variants in G6PC3 (c.210del, p.Phe71Serfs*46), establishing a molecular diagnosis that matches the clinical presentation of this patient. Interestingly, repeat heme-STAMP testing on peripheral blood and bone marrow 12 months after the initial studies no longer detected the KRAS-mutated clone.

Management: Upon diagnosis of a congenital neutropenia syndrome, the patient was tapered off steroids while G-CSF treatment was continued. Cardiac echo and abdominal/pelvic ultrasound will complete the syndromic work-up. Pros and cons of a hematopoietic stem cell transplant are currently being evaluated.

Conclusion: Our experience suggests that a diagnosis of congenital neutropenia due to G6PC3 may not be straightforward in patients with neutropenia and thrombocytopenia. A high index of suspicion and other syndromic features of G6PC3 may be clues to the diagnosis. Screening of all combined immune deficiencies including neutropenia may help to uncover the whole spectrum of patient with G6PC3 deficiency.

(213) Submission ID#812526

Five-Year-Old Boy with Very Early Onset Inflammatory Bowel Disease

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Abstract/Case Report Text

The patient is a five-year-old fully immunized boy who presents for immunological investigation of inflammatory gastritis and enteritis. He was previously healthy until age three, when he presented to an outside facility with lower extremity edema and hypoalbuminemia. Endoscopy revealed gastritis and duodenitis with grossly normal esophagus and colon, resulting in a diagnosis of protein losing enteropathy. Elimination diet was attempted with variable adherence.

He continued to follow in gastroenterology clinic with ongoing abdominal complaints including intermittent abdominal pain, abdominal distention, vomiting and alternating constipation and diarrhea without blood. At initial presentation, his weight was at 14% and height at 25%, but this has decreased over two years to weight < 1% and height < 5%. Additional past medical history is notable for a documented episode of transient left ankle swelling at age 44 months. There is no history of fevers, sinopulmonary infections, skin lesions, invasive bacterial infections, mucocutaneous candidiasis, recurrent viral infections or endocrine abnormalities. The family is of African-American and Congolese ancestry. The patient has no siblings, both parents are healthy and there is no family history of immune disorders, autoimmunity or inflammatory bowel disease.

Additional studies revealed a normal white blood cell count with elevated monocytes, normal hemoglobin, platelets >1000 x 109 cells/L, persistent hypoalbuminemia (1.4-2.8 g/dL), elevated C-reactive protein (20-51 mg/L), intermittently elevated erythrocyte sedimentation rate (12-44 mm/h), negative tissue transglutaminase IgA and consistently elevated fecal calprotectin and alpha-1-antitrypsin. Initial immune evaluation had revealed normal dihydrorhodamine flow cytometry; elevated IgE (822) but normal IgM, IgA, IgG and IgD; and normal T, B and NK cell numbers (CD19+ 737 cells/μL, CD4+ 1441 cells/μL, CD8+ 376 cells/μL, CD16/56+ 390 cells/μL).

Over the following two years, he underwent two additional esophagogastroduodenoscopies and colonoscopies, which have repeatedly shown ulcerations of the stomach and duodenum with normal esophagus and colon. Biopsies of the duodenum in at ages 3 and 4 showed duodenal mucosa with villous blunting, crypt hyperplasia, expansion of the lamina propria, neutrophilic inflammation, intact goblet and Paneth cells, detectable plasma cells and absent intraepithelial lymphocytosis. CT revealed hepatosplenomegaly. CT enterography revealed mild small bowel wall thickening and mucosal hyperenhancement.

Based on clinical history and studies, very early onset inflammatory bowel disease was suspected. Enteral budesonide was attempted, but patient had difficulty with adherence. He completed a 5-week course of prednisolone with temporary improvement in symptoms but recurrence after steroids were tapered. Due to the concern for a genetic cause of early onset inflammatory bowel disease, whole exome sequencing was performed of proband and both parents. This revealed a paternally inherited heterozygous pathogenic mutation in mevalonate kinase (MVK; p.Y116H) and a maternally inherited variant of uncertain significance in Inducible T Cell Costimulator (ICOS; p.V151L). Additional functional testing is underway to understand whether this patient's variants may play a role in disease pathogenesis or whether additional as-yet unidentified genetic variants may be responsible for his immune disorder.

(214) Submission ID#812527

Gain-of-Function Mutations in STAT1: A Novel Manifestation with Pulmonary Granulomatous Disease

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Abstract/Case Report Text

Introduction Signal transducer and activator of transcription (STAT1)1 gain of function (GOF) mutations result in increased STAT tyrosine phosphorylation and secondarily increased response to STAT1-signaling cytokines, such as interferons determining defective Th17 cell development and subsequent susceptibility to infections and, in many cases, autoimmune manifestations.

Clinical manifestations of patients with STAT1 GOF are characterized by disseminated or recurrent infections due to s. aureus, herpes viruses,

mycobacteria and fungal infections, characteristically presenting as chronic mucocutaneous candidiasis. In addition to infectious susceptibility many patients have autoimmune manifestations including hypothyroidism, thyroiditis, type 1 diabetes mellitus and immune cytopenias. There is no clear correlation between STAT1 domain-specific mutations and phenotype, and it remains unclear why GOF mutations in STAT1 result in such a wide spectrum of clinical presentations.

Case Report Three year old female born to non-consanguineous parents at 3 years of age is admitted to the hospital for workup of an insidious 4 month episode of persistent mainly facial and upper limb edema associated to respiratory symptoms including cough and dyspnea. On her medical history she had one previous episode of oral candidiasis, one episode of pyelonephritis at 11 months and one otitis media. On exam, she had normal height and weight for her age remarkable facial edema, pulmonary crackles, oral thrush and multiple, small cervical, axillae and inguinal lymphadenopathies and a palpable spleen. High resolution chest CT showed multiple bilateral parenchymal nodules and confirmed multiple adenopathies. An abdominal US identified mild hepatosplenomegaly. Bronchoalveolar lavage fluid was positive for S. maltophilia and multiple copies of EBV; there was no evidence of fungi, parasites, or mycobacteria. A cardiac ultrasound evidenced mild pericardial effusion. Biopsy of pulmonary nodules was suggestive of lymphomatoid granulomatosis and biopsy of the pericardium showed nonspecific chronic inflammation. EBV was positive in lung tissue and pericardial fluid. Tuberculin skin, mycobacterial cultures in BAL and interferon-gamma release assay (IGRA) were negative reasonably ruling out mycobacterial infection. Immune workup showed normal blood counts, immunoglobulins and lymphocyte populations within normal ranges. Additional autoantibody testing (ANA, ENA, ANCA) were negative. A primary immunodeficiency was suspected and Genetic testing (Invitae PID panel) revealed a previously associated to gain of function, heterozygous mutation in STAT1 c.820C>T (p.Arg274Trp). Both parents were negative for this mutation indicating it occurred de novo. Intravenous immunoglobulin (IVIG) replacement and prophylactic antibiotics were started. Lymphomatoid granulomatosis in the setting of persistent EBV infection was treated with 2 doses of Rituximab with excellent response and clearance of pulmonary nodules and respiratory symptoms.

Conclusions

We present the first Chilean patient with STAT1 GOF immunedysregulation . Moreover, to our knowledge this is the first STAT1 GOF patient presenting with lymphomatoid granulomatosis. This is a severe pulmonary disease in which primary immunodeficiencies including STAT1 GOF should be considered in the differential. In this case Rituximab successfully resolved pulmonary nodules and respiratory symptoms.

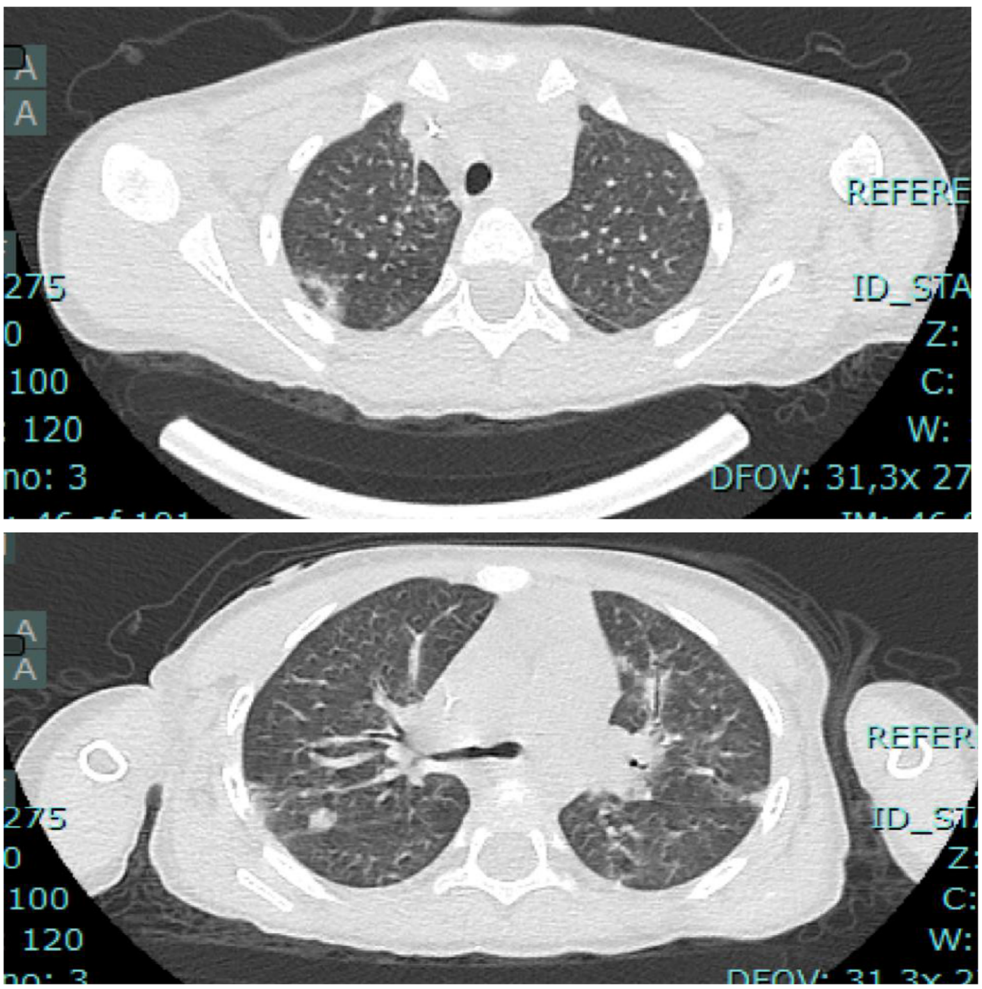

Figure 1. Hi resolution Chest CT showing multiple bilateral pulmonary nodules and small bronchiectasis.

(215) Submission ID#812530

Use of recombinant human IL-18 Binding Protein In A Pediatric Patient With X-Linked Lymphoproliferative Disease Type-2

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Abstract/Case Report Text

A 14 year-old male with a history of XLP-2 was diagnosed at age 4 after EBV induced HLH. A four base deletion mutation was identified in the XIAP/BIRC4 gene. He was evaluated for consideration of bone marrow transplantation, the decision for which was ultimately deferred due to both lack of HLA matched donors and anecdotal report of poor outcomes for HSCT in XIAP patients. The patient recovered from his first HLH. There was persistence of mild hepatosplenomegaly but otherwise clinical stability and monitored expectantly until the age of 12. At that time he presented with fever, left knee and ankle arthritis. He underwent arthrocentesis of the left knee and left ankle, both aspirates were sterile, with notable leukocytosis with heavy neutrophilic predominance. An extensive rheumatologic and infectious workup was non-diagnostic. Both sIL-2R and IL-6 were elevated, 5,026 units/mL (< 1105) and 44pg/mL (< 6), respectively. He was treated with systemic corticosteroids, ultimately arthritis resolved after 2 months. At age 13 he presented for the first time with periorbital pain and conjunctival injection of the left eye that persisted after minor trauma. He was found to have non-granulomatous uveitis, which responded ultimately to systemic corticosteroid. He then presented at age 14 with fever and right knee and great toe arthritis. Again he underwent arthrocentesis which revealed aseptic arthritis, and at that time was started on Anakinra (anti IL-1β) and prednisone. There was clinical improvement over several weeks followed by return of right knee arthritis, coupled with onset of symptomatic uveitis of the left eye. Despite systemic corticosteroids and Anakinra and IL-1 blockade, the patient was again admitted shortly thereafter to the hospital with arthritis, fevers, rash and abdominal pain. There was concern for evolving HLH and the patient was ultimately transferred to Cincinnati Children's for further evaluation and treatment. Pertinent inflammatory biomarkers at that time included sIL-2R of 3,394 units/mL and IL-18 level of 9,624 pg/mL. In addition to Anakinra and systemic corticosteroids, the patient was started on Tadekinig alfa (recombinant human IL-18 binding protein) as part of a prospective study. HLH flare ultimately resolved without use of antineoplastic agents, and the patient was discharged home. Soluble IL-2R levels since normalized, and IL-18 levels decreased to less than 1000 pg/mL. The patient has been doing well on Anakinra and Tadekinig alfa, though continues to experience mild to moderate right knee effusion. This case suggests IL-18 inhibition may be an effective therapeutic approach for patients with XIAP deficiency.

(216) Submission ID#812531

Development of a Clinically Validated Test for Dendritic Cell Enumeration

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Abstract/Case Report Text

Dendritic cells (DCs) are a group of circulating and tissue resident cells that play a critical role in both innate and adaptive immune responses. DCs include two major subsets: myeloid (or classical) and plasmacytoid, mDC and pDC respectively. These subsets perform distinct in vivo functions and promote different effector functions in response to pathogens. mDCs can capture and present antigens to CD4+ T cells and cross-present them to CD8+ T cells. They are also a source of inflammatory cytokines. pDCs take part in priming of anti-viral T cells and are the major source of type I interferons.

DC deficiency is a diagnostic component of several genetically defined immunodeficiencies, including GATA2 deficiency, IRF8 deficiency, STAT3 GOF, IKZF1 deficiency, HYOU1 deficiency, reticular dysgenesis due to AK2 mutations and WHIM syndrome. In addition, unexplained monocytopenia can be a relevant clue in detecting DC deficiency. Currently, the full range of immune defects caused by DC deficiency is not established, but the availability of a clinically validated assay can help mitigate the diagnostic challenge.

To address this diagnostic need, we have developed a single platform test to enumerate mDCs, pDCs and monocytes. The method consists of a whole blood no wash assay with a laboratory-developed polychromatic monoclonal antibody panel. Addition of flow count beads allows us to report the absolute count of each evaluated population per microliter of blood. In this panel, pDCs are defined as CD45+, Lin2neg, HLA-DR+, CD123hi; mDCs as CD45+, Lin2neg, HLA-DR+, CD11c+; and monocytes as CD45+, CD14+-low. Lineage 2 (Lin2) consists of CD3, CD14, CD19, CD20 and CD56. Isotype controls to HLA-DR, CD11c, and CD123 are included in this 4-tube test. A daily in-house normal donor is included on each run.

In order to establish a reference range for the assay, mDC, pDC and monocyte enumeration was performed on blood obtained from 227 unique normal donors. This group included 164 adults between 18-78 years of age and 63 pediatric donors from 4 months to 17 years of age. The age of the donors was distributed across the decades of life. Within the adult population, 52% were female, whereas 60% of the pediatric recruits were female. Reference ranges were calculated using quantile regression methods with a 95% confidence interval on the middle 95% of the range.

We have confirmed the clinical utility of this test by detecting several patients with established primary immunodeficiency diagnoses.

We believe that the availability of this clinically validated test at a US clinical reference laboratory will be beneficial in identifying patients with potential immunodeficiencies involving dendritic cells and/or monocytes.

(217) Submission ID#812544

Successful Management of Primary Hemophagocytic Lymphohistiocytosis by Ruxolitinib, a JAK Inhibitor

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Primary hemophagocytic lymphohistiocytosis (HLH) is a life-threatening hyper-inflammatory disease mainly caused by defects in genes of the granule cytotoxic pathway, inducing extreme inflammation and massive tissue infiltration by activated T cells and macrophages. Hematopoietic stem cell transplantation (HSCT) is the only available curative treatment with a an overall survival of 60-70%. As success of HSCT is dependent on complete control of the disease prior to transplantation, the development of targeted and more potent anti-inflammatory treatments would be a major advance in the treatment of HLH. More recently, preclinical studies in HLH murine models demonstrated therapeutic efficacy of ruxolitinib, a Janus Kinase (JAK)-1/2 inhibitor, on HLH manifestations (1-3). Ruxolitinib is licensed to treat hematological malignancies and its pharmacokinetic has already been documented in the pediatric population.(Loh Pediatrr Blood Cancer 2015) In June 2019, we evaluated a febrile 15-month old girl for a suspicion of incomplete Kawasaki disease. She presented with 5 criteria of HLH: (i) prolonged fever > 38.5° C, (ii) splenomegaly, (iii) profound pancytopenia, (iv) increased triglyceride and reduced fibrinogen levels, (v) hemophagocytosis in her bone marrow biopsy; but only modestly elevated ferritin levels (less than 500 ·g/L). Lumbar puncture and brain MRI were negative. Flow cytometry revealed the presence of activated HLA-DR+ CD8 T cells and the absence of perforin expression by CD8 T and NK cells. Perforin deficiency was confirmed genetically with the presence of two rare variants in *PRF1* (c.445G>A and c.116C>A). She was initially treated with solumedrol (1-2 mg/kg/day) which corrected the anemia, thrombocytopenia and normalized the CRP. In the absence of neurological involvement and infectious trigger, ruxolitinib was initiated at a dose of 50 mg/m^2/day, in combination with dexamethasone (10 mg/m^2/day). This treatment led to rapid normalization of the neutropenia (48 hours), complete resolution of the splenomegaly (10 days) and disappearance of HLH biological markers (triglycerides levels in 1 week, activated HLA-DR+ CD8 T cells in 2 weeks, fibrinogen levels in 1 month), without the need for etoposide or serotherapy. Dexamethasone was weaned every two-weeks and stopped after 8 weeks. Ruxolitinib was well-tolerated with no side effects. While in complete remission of her HLH, the patient then received alemtuzumab (0.5 mg/kg total dose) and a fludarabine-based myeloablative conditioning regimen. Ruxolitinib was weaned over one week, and a 9/10 unrelated transplant was performed with success. The immediate post-transplant period was complicated by a veno-occlusive disease that responded rapidly to defibrotide and a corticosteroid-resistant skin and ocular graft-vs-host disease (GVHd) despite a prophylaxis with ciclosporine and mycophenolate mofetil. GVHd was controlled by the reintroduction of ruxolitinib. At 3 months post-HSCT, her chimerism is 100% donor. To our knowledge, this case is the first description of a patient with primary HLH successfully treated in first intent by a combination of dexamethasone and ruxolitinib prior to HSCT. Our observation suggests that this targeted and less-toxic treatment regimen, that does not include etoposide nor high-dose alemtuzumab, is effective, well-tolerated and could be used in first intent to treat primary HLH.

Acknowledgments: We want to thank Bénédicte Neven, Necker-Enfants Malades, France and Kim Nichols, St Jude Children's, USA for fruitful discussion; and the HSCT team of the CHU Ste-Justine.

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(218) Submission ID#812546

Diagnostic Yield of Whole-Exome Sequencing For 55 Patients with Primary Immune Deficiencies

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Abstract/Case Report Text

INTRODUCTION: The National Institute of Pediatrics (INP) is a national referral center for PID diseases (PIDD). At the Immunodeficiencies Research Unit we discuss and pursue the molecular and genetic diagnoses for patients with suspected PID from all around the country. Starting this year, we are processing and analyzing our own patients' WES results at the Unit. Evaluating the diagnostic yield of WES, as a measure of effectiveness or quality control, may result in process optimization and perhaps allow for better patient selection and resource allocation.
OBJECTIVE: To describe and characterize our patients with suspected PIDD whose DNA samples were sent out for whole-exome sequencing; to analyze and compare our WES diagnostic yield after the first 3 batches of patients; to identify patient attributes that may predict a positive diagnostic WES result.
METHODS: Genomic DNA was obtained from whole-blood samples of patients with suspected PIDD from hospitals in Mexico City, Monterrey and Puebla. WES was performed using a NG sequencer (Illumina HiSeq) in New Jersey (Admera Health, LLC), with 90% coverage and a 50x depth of the IDT Xgen library, Human genome version 38 (December 2013). Two FASTQ files for each patient sample were transferred back to our Unit, where the bioinformatic workflow was completed. We used Galaxy in the cloud for quality control, mapping & alignment, and detection of variants; Variant Effect Predictor to process, map, annotate and filter variants; and IGV (Broad Institute) and Genome browser (UCSC) for visualization. We defined diagnostic yield as the proportion of patients with a genetic diagnosis after analysis of their WES results. We performed multivariate logistic regression, tree partitioning algorithm and linear

discriminant analysis to explore differences between diagnosed and undiagnosed cases.
RESULTS: A rare, pathogenic variant in a gene known to cause a PIDD that matched the patient's clinical and laboratory phenotype was found in 23 of the 55 exomes, for a diagnostic yield of 42%. Explorations with multivariate linear regression (MLR), linear discriminant analysis (LDA), and tree partitioning algorithm (TPA) suggested two attributes nearing statistical significance: Family affected, and Dysgammaglobulinemia. 14 studies that matched their inclusion criteria, in which the diagnostic yield ranged from 15 to 79%. The percentage of patients who were genetically diagnosed by NGS in mixed PID groups (our case) ranged from 15 to 46%, with a median of 25%.
DISCUSSION: WES is an effective diagnostic tool. Definitive genetic diagnoses, here and elsewhere, impact the management, counseling, classification and epidemiology of rare diseases including PIDD. Here, we describe the results of our first batches of patients sequenced and analyzed in-house.

(219) Submission ID#812548

Monogenic Diagnoses of Adolescents and Adults at A Primary Immunodeficiency Transition Clinic in Vancouver, Canada

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Abstract/Case Report Text
Rationale: Primary immunodeficiencies (PIDs) are a group of genetic disorders with heterogeneous clinical manifestations affecting both children and adults. Adults with PIDs face high morbidity and mortality, and the period of transitioning care from pediatric to adult providers is a particularly vulnerable time for those with chronic illness. Improved access to genetic sequencing has the potential to improve outcomes through treatment tailored to the patient's specific molecular defect. Recognizing the unique needs of this population, a PID transition clinic was established in Vancouver, Canada in 2018. Staffed by pediatric and adult immunologists, the clinic serves adolescents transitioning into adult care, as well as adults with suspected or known PIDs. We sought to determine the frequency of monogenic PIDs and the impact of genetic diagnosis on management for patients seen in this clinic.
Methods: A retrospective chart review was performed of the patients who attended the PID transition clinic between March 2018 and September 2019. Baseline characteristics, diagnoses of patients who underwent genetic sequencing, and the impact of a genetic diagnosis on the patient's treatment plan were evaluated.
Results: The charts of 54 patients were reviewed, 24 of whom had received genetic sequencing for PID in a clinical lab facility. Seven of the 24 patients who underwent sequencing were diagnosed with a monogenic PID. The median age at confirmatory genetic diagnosis was 20 years (range prenatal to 55 years), with 5 patients diagnosed at age 17 or greater. There was a mean latency of 16.1 years between symptom onset and the age at genetic diagnosis (range = 0-34 yrs), with family member testing accounting for 2 patients without latency of symptom onset. There were 17 patients in whom a diagnosis was not confirmed by sequencing: 11 had variants of uncertain significance, 2 had variants confirmed benign through functional testing, and 4 patients had negative testing results. The monogenic forms of PID seen in adolescent and adult patients included ADA2 deficiency, chronic granulomatous disease, X-linked neutropenia, GATA-2 deficiency, X-linked agammaglobulinemia, ALPS-FAS, and STAT3 loss-of-function. Sequencing results led to changes in treatment plans in 7/7 patients, including referral for hematopoietic stem cell transplantation (N=1), targeted therapy (N=1), and disease-specific antimicrobial/immune prophylaxis and/or monitoring (N=7).
Conclusions: Transition clinics allow continuity of care for adolescent PID patients, as well as reassessment of patients with clinical histories suspicious for PID throughout adulthood. We identified a long latency period between age at symptom onset and age at confirmatory genetic diagnosis of patients with monogenic PIDs. It is critical to consider genetic testing in patients of all ages with histories suspicious for a PID, as there is a high diagnostic yield and impact on management in this population.

(220) Submission ID#812550

Ulcer of Lipschütz within Hyper IgD syndrome, A Rare Combination

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Abstract/Case Report Text
Presentation A 15-year-old girl, presented in ambulatory consultation, with a 3-year history of recurrent fever, influenza-like symptoms (sore throat, malaise), associated with self-limited painful genital ulcers (just within the period of fever). The first episode was characterized for an Fournier's infection, requiring in-hospital treatment, multiple surgical procedures, antibiotics and hyperbaric oxygen therapy. After that catastrophic debut, she was diagnosed approximately 6 episodes per year pharyngitis (with fever, malaise and sore throat) treated with corticoids, antibiotics and topic medication. The last year, noticed that every episode of fever (total of 4) were associated with one or several genital lesions. The patient had no relevant medical history, she didn't receive long-term medication, she received all immunizations, she was sexually inactive, and hadn't apply any topic medication or product on the vulva, there was no trauma history, psychological medical history or sexual abuse. Episodic gynecologic examination showed her labia minor several lesions, fibrinous, soft ulcerations on their inner aspect, these lesions had a symmetrical appearance, known as kissing lesions; no vulvar swelling, vaginal discharge or lymphangitis were noticed. There were no other skin or mucous membrane lesions (Figure 1).
Investigations
Viral (HIV, HBV, HCV, EBV, CMV) and treponemal (TPHA-VDRL) serologies were negatives. Erythrocyte sedimentation rate (ESR) and PCR analysis within ulcers episodes were positive. Specific antibodies (Cardiolipin, Anti RO/SS-A, Anti LA/SS-B, Anti CCP, Anti ENA, Anti Gliadin, Anti TPO Anti TPO) serologies were negative. Otherwise, important elevation Immunoglobulin D was observed (9,3 mg/dL, twice the normal value): Mild elevations of Immunoglobulin M and Immunoglobulin A were observed. Serum subtypes of immunoglobulin G and immunoglobulin E were normal. Leukocytosis with monocytes elevation and an increase of Lymphocytes B were present. (Table 1).
Discussion Lipschütz ulcers are uncommon and an often unknown entity for physicians, but it is important to recognize and include it in the differential diagnosis of vulvar ulcerations. This condition is characterised by self-limited painful ulcerations of the vulva or lower vagina in adolescent or young women, non-sexually transmitted, and usually preceded by influenza or mononucleosis-like symptoms.

Hyperimmunoglobulin D syndrome (HIDS) is characterized for unremitting fever lasting four to seven days and the presence of palpable tender lymphadenopathy, splenomegaly, arthralgia/arthritis, abdominal pain, and mucocutaneous manifestations. Laboratory findings suggestive of HIDS include elevated age-specific serum immunoglobulin D (IgD) and/or immunoglobulin A (IgA) levels, elevation of acute phase reactants, and urinary excretion of mevalonic acid during, but not between, attacks. The diagnosis is established if an elevated age-specific level of IgD is detected. IgA levels are typically measured at the same time but are not required for diagnosis. Elevated serum IgD is not specific for HIDS and can occur in patients with certain neoplastic, infectious, heritable, and idiopathic disorders.

In the present case report, the patient was treated with colchicine, with favorable evolution and free from new events. Levels of Ig D, platelets and monocytes remain high.

Conclusion

We describe a young female patient presenting recurrent Lipschütz ulcers, fever and elevation of serum immunoglobulin D, suggesting that HIDS could be associated with genitalia ulcers.

(221) Submission ID#812552

Long Term Use of Holder Pasteurized Donor Breastmilk in A Premature Infant with SCID/Athymia, Without CMV Transmission

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Abstract/Case Report Text

This is an 8-week old male infant born at 29 weeks, birthweight 1390 g. Mother is 17 years old, G1P1 with history of type 1 diabetes, poorly controlled throughout pregnancy. Pregnancy was additionally complicated by antepartum hypertension, but not by infection. Prenatal ultrasound on the day of delivery indicated hypoplastic left ventricle. Echocardiogram after delivery demonstrated single ventricle with type I truncus arteriosus. He was also noted to have cleft palate, solitary right kidney, and hypocalcemia. Newborn screen on day 3 of life noted undetectable TRECs. Per the screening algorithm in our state, this undetectable result was followed by flow cytometry which showed CD3 positive cells at 0.5% of total lymphocytes (13/mcL). CD19 positive lymphocytes were 75% (1836/mcL), and CD16/56+ cells were 21.3% (519/mcL). Chromosomal microarray normal. A next generation sequencing panel encompassing 105 genes did not identify a primary immunodeficiency. The child was initially at an outside facility and was being fed with pasteurized donor breast milk. Mother's breastmilk had never been used. The facility was instructed to discontinue donor breastmilk feeds and switch to formula, and preparations were made to transfer the child to our facility. 24 hours after feeds were changed, the child developed vomiting, bloody secretions per NG tube and increasing lactate. Abdominal x-ray showed pneumatosis and portal venous gas. He was diagnosed with necrotizing enterocolitis (NEC). All enteral feeds were stopped. He completed a course of broad-spectrum antibiotic. Surgery was not required for his NEC. He was started on IV immunoglobulin, Bactrim, and fluconazole prophylaxis.

With resolution of NEC, discussions began about re-initiation of enteral feeds. Interestingly, despite having had donor breastmilk at the outside facility, viral studies including CMV PCR were negative on admission and remained negative throughout his course to that point. Maternal CMV serologies were positive, thus her milk could not be used. Literature was reviewed on the risk of NEC with donor breastmilk versus non-breast milk-based formulas(1), and the safety of donor breast milk was considered in detail. Processing of donor milk used at our institution is performed according to HMBANA guidelines, which include Holder pasteurization (62.5 °C for 30 minutes). Literature on the effectiveness of Holder pasteurization in eliminating CMV infectivity in vitro(2), and transmission in vivo in extremely low birth weight infants was considered(3). Based on these considerations, the risk/benefit was considered favorable for restarting pasteurized donor breastmilk feeds. Given his small size and young age, we had significant concerns about using ganciclovir prophylaxis and opted to hold this and monitor weekly CMV PCR. The child has been titrated up on these feeds, is gaining weight appropriately, and has had weekly CMV PCRs which are negative x5 since restarting donor breastmilk. T cells, last checked at 7 weeks of age (37 weeks gestational age) remain essentially absent. Given the lower risk of NEC in premature infants with breastmilk-based enteral feeds, a broader, multi-institutional study is warranted to best examine the safety of pasteurized donor breastmilk in infants with SCID and complete DiGeorge syndrome.

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(222) Submission ID#812554

The Costa Rican Registry For Primary Immunodeficiencies (1985-2019)

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Abstract/Case Report Text

The Costa Rican PID registry was established in 1985. The aim of the registry is to collect data on epidemiology, molecular diagnosis, mortality, malignancy and treatment from PID cases diagnosed in Costa Rica.

Methods: We analyzed the registered data from 1985 to 2019 and reported the prevalence of active patients during the year 2019. The study was approved as CEC-HNN-024-2017.
Results: Costa Rica has a population of five million people (30% with an age below 19 years). In 2011, the Costa Rican PID represented 6% of reported cases among Latin-American countries.
The overall prevalence of PID in Costa Rica is 3.3/100.000 inhabitants (160 PID). Ataxia-Telangiectasia is the most prevalent (0.98, n=97), followed by antibody deficiencies (0.72). During the period of the study, 298 PID were registered (55% males). The distribution according to phenotype was CID 13% (n=38), CID with Syndromic Features 54% (n=161, AT 132), Antibody Deficiencies 13% (n=38, XLA 15), Immune Dysregulation 2% (n=6), Neutrophil Defects 4% (n=12, CGD), Osteopetrosis 13% (n=38), other 4% (FMF 1, C1qD 3). Fifty-five percent of the patients are alive. The highest mortality (68%) was observed in the CID group. HSCT was done in 6 cases with Reticular Dysgenesis, 9 SCIDs, 1 ADA deficiency, 1 CD40L-, 2 FLH-4 (syntaxin 11 deficiency), 1 IPEX and 8 Osteopetrosis. Nineteen patients developed malignancies, mainly non-Hodgkin lymphomas among AT patients (74%). Other malignancies were a case with Buschke Lowenstein in a DOCK8 deficiency, a hepatocellular carcinoma in a CD40L-, and a lymphoma in some of the FHL-4, Chediak Higashi and PI3KRI cases.
SCID was registered in 9.8% of all PID. Costa Rica does not have newborn screening for SCID. Twenty-eight percent of SCID patients were Reticular Dysgenesis, which form part of the same indigenous, consanguineous family. Two of them died before an HSCT was considered, 6 received HSCT and 2 died one-month post-transplant (HSCT done at age older than 3 months). Four patients of this family were successfully transplanted and are alive.
Among the other SCID cases, one was an ADA deficiency and 20 had an unknown molecular defect. Sixteen patients died, 9 received HSCT (1 before 3 months of age). The first SCID was transplanted in 1985. Among the SCID patients, 15 had an HSCT and 8 are alive (47% mortality). AT is the most common PID in Costa Rica and it represents 44% of the patients registered. Malignancy presented predominately in AT patients as expected. It was distributed as 8 non-Hodgkin lymphomas, 1 gastric adenocarcinoma, 2 nasopharyngeal NHL, 1 T-cell lymphoma, 1 ovarian cancer and 1 hepatoblastoma. Two AT patients had 2 malignancies (non-Hodgkin and Hodgkin lymphoma). Osteopetrosis is also very prevalent (0.48). Mortality decreased since HSCT is performed using an haploidentical protocol with mothers as donors (12% mortality).
Conclusions: During the study period, 298 PID were registered, mainly AT and Osteopetrosis cases. SCID represented 12% and XLA 11% of patients. Treatment included HSCT, restitution with IVIg, and prophylaxis with TMP/S and Itraconazole. Malignancy was 6% and 55% of cases are alive.

(223) Submission ID#812556

Intralesional Corticosteroids As Adjunctive Therapy For Refractory Cutaneous Lesions In Chronic Granulomatous Disease

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Abstract/Case Report Text

A 15-year-old male with X-linked Chronic granulomatous disease (CGD) complicated by severe perianal disease and proctocolitis presented with two weeks of open draining lesions on the thighs, bilateral inguinal regions, and gluteal cleft. The wounds became excruciating and prevented normal ambulation. The patient was admitted for IV antimicrobial therapy, local wound care and systemic steroids. Wound cultures, throughout his course, yielded growth of Klebsiella pneumonia, Candida parapsilosis, Malassezia globose, Escherichia coli, Enterococcus faecalis and Staphylococcus epidermidis allowing for directed antibiotic and antifungal therapies. Despite improvement, the wounds persisted after several weeks of treatment.
Faced with recalcitrant cutaneous lesions despite aggressive systemic and topical therapies, we looked to alternative options. Noting that other granulomatous diseases show response with intralesional corticosteroid therapy, we considered this for our patient (1, 2). For example, patients with idiopathic granulomatous cheilitis had a complete response after three monthly injections of intralesional corticosteroids (3). Sarcoidosis patients also improve with intra-granuloma corticosteroid injection (4,5). Our patient received 20 mg triamcinolone acetonide injections two separate occasions, administered in multiple open lesions at eight-week intervals. The cutaneous lesion improvement was gradual and complete resolution of the first open wound was noted fifty-two days from initial steroid injection.
To our knowledge, intralesional glucocorticoid therapy has not previously been used to treat cutaneous disease in CGD patients. We are reporting the first CGD patient with successful lesion resolution following steroid injection as part of therapy. As such, we believe this case is significant and suggests that direct lesion injection with glucocorticoids can add to treatment options for CGD patients with recalcitrant cutaneous disease.
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Immunological Features Associated With Mono- And Biallelic Transcription Factor 3 (TCF3) Null Mutations: A Gene Dosage Effect

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Abstract/Case Report Text
Transcription factor 3 (TCF3) monoallelic/dominant negative (DN) mutations affecting E47 but not E12 (the two isoforms of TCF3 protein) were originally described in patients with absent/low B cells and agammaglobulinemia. Later, a few single patient/family case reports described biallelic/loss of function mutations in TCF3 with absent/low B cells, hypogammaglobulinemia and B-ALL. Herein we report an extended in-depth analysis on immune and clinical penetrance associated with mono- and biallelic TCF3 null mutations.
We studied 3 families (A, B and C) with novel null mutations in TCF3 (NM_003200). Family "A" consists of three children (A.II.1; 6 y.o./M, A.II.2; 3 y.o./M and A.II.3; 8 mo/F), carrying a biallelic branch point mutation (c.1451-18 A>T; p.G486Lfs*4), causes addition of 11 intronic nucleotides followed by frameshift, inherited from consanguineous parents (A.I.1 and A.I.2). Family "B" includes 1 patient (B.I.1; 10 y.o./F) with a de novo monoallelic nonsense mutation (c.1541 C>A; p.S514*). Family "C" involves an adult patient (C.I.1; 53 y.o./F, familial segregation unknown) carrying a monoallelic insertion leading to early protein termination mutation (c.599delinsGT; p.Y200Sfs*54).
Clinical manifestations included upper and lower respiratory infections in 2/7 mutation+ individuals; a monoallelic patient (C.I.1) presented with recurrent sinusitis, and a biallelic patient (A.II.1) presented with recurrent infections and B-ALL. Rest of the mutation positive individuals were clinically asymptomatic.
B cell immunophenotyping in the 7 mutation+ individuals (3 bi- and 4 monoallelic) showed low B cell numbers in 5/7 individuals (3 bi- and 2 monoallelic) while normal on other two monoallelic patients (A.I.1 and C.I.1), and reduced class switched memory B cells were observed in all. Serum IgG, alone or in combination with IgA and/or IgM, were reduced in all patients. T-follicular helper cells were reduced only in patients carrying biallelic mutations (A.II.1, A.II.2 and A.II.3) but not in any patients with monoallelic TCF3 null mutation. T cell enumeration and function by means of proliferation was normal in all mutation+ individuals.
No mutated (truncated) protein expression was detected from patients with either biallelic or monoallelic TCF3 null mutations. However, wildtype TCF3 protein was detectable in about half amount in heterozygous patients. cDNA data showed either 0/100 or 50/50 WT/mutated transcripts ratios in homozygous or heterozygous individuals, respectively, suggesting mutated proteins instability; and all together, protein haploinsufficiency for the heterozygous cases.
Ex-vivo, CD40L and IL21-induced plasmablast differentiation was found to be reduced in 4/5 patients tested (1 biallelic patient A.II.2 and 3 monoallelic patients A.I.2, B.I.1 and C.I.1). Moreover, decreased IgG, IgA and IgM production in vitro correlated with reduced plasmablast cell differentiation.
In conclusion, all individuals carrying either mono- or biallelic null mutations have immunological penetrance of the B cell defect. However, while clinical penetrance was complete in patients with biallelic mutation, it was partial for those with monoallelic TCF3 null mutation suggesting a gene dosage effect for clinical penetrance. In addition, our study emphasizes that TCF3 is relevant to the plasmablast differentiation process as well as for Ig production. Further studies are being conducted to evaluate the individual roles of E12 and E47 on the immune and clinical features.

(225) Submission ID#812566

Impact of Novel STAT1 Gain-of-function D292E and K388Q mutations on the NK cell function

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Background: Heterozygous gain-of-function (GOF) mutations in the *STAT1* gene result in a hyperphosphorylated state where patients develop recurrent or persistent chronic mucocutaneous candidiasis (CMC), other cutaneous mycosis, bacterial infections, disseminated dimorphic fungal infections and autoimmune disease. Furthermore, the NK defect we characterized illustrated an immature $CD56^{dim}$ NK cell subset with decreased expression of CD16, perforin, CD57, and impaired cytotoxic capacity associated with increased susceptibility to viral infections observed in these patients.
Methods: In this study, we evaluated 2 patients with novel *STAT1* mutations (D292E mutation, located in Coiled-coil domain and K388Q mutation, located in DNA-binding domain). A third patient with the previously reported V266I mutation (CCD) was also recruited for this study. *In vitro,* PBMCs from these patients were stimulated with IFN-α for 30, 60, and 120 minutes and levels of phospho-STAT1 on $CD56^{dim}$ NK cell subset were measured by flow cytometry. The STAT1 activity (firefly and Renilla luciferase activities) was evaluated in U3A-STAT1 deficient cells

transfected with a reporter plasmid (for luciferase), WT or mutant-STAT1 plasmids. NK cell cytotoxicity was measured by Cr^{51} release assay. We used multiparametric immune profiling to dissect the effect of *STAT1-GOF* mutations on NK cell developmental phenotype.

Results: Similar to our previous studies, we observed higher levels of STAT1 phosphorylation after two hours of stimulation from the DBD mutation compared to the CCD mutations. The STAT1 activity assay confirmed gain of function observed by flow cytometry, but this activity was higher in K388Q mutant and D292E mutant (CCD-closer to DBD) than V266I mutant. All patients demonstrated low NK cell lytic unit compared to healthy donors. Interestingly, we observed a correlation between low lytic unit and lower numbers of $CD56^{dim}Perforin^{+}CD16^{+}$ NK cells; much lower in patient with K388Q mutation.

STAT1-GOF patients showed a significant decrease in total NK cell numbers and impaired NK cell maturation was characterized by low expression of CD57, and higher levels of immature NK cell markers (CD117, NKG2A, CD158b).

Conclusions: These data suggest that impairment of NK cell function is affected by the location of the *STAT1* mutation and continues to be the case in novel mutations identified. The identification the genotype/phenotype correlation in the spectrum of the NK cell defect in *STAT1* gain-of-function mutants may help to better understand the molecular basis for STAT1 activation and/or function to predict clinical manifestations of disease and ultimately treatment regimens.

(227) Submission ID#812574

Jacobsen Syndrome as a Cause of Abnormal TRECs on Newborn Screen: Immunophenotypes in a Terminal 11q Deletion Syndrome Cohort

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Abstract/Case Report Text

Background: T cell lymphopenia associated with genetic syndromes can be identified with low T cell receptor excision circle (TRECs) up on the newborn screen for Severe Combined Immune Deficiency (SCID). Jacobsen syndrome (JS), 11q terminal deletion, is a rare genetic disorder seen in 1/100,000 births characterized by facial dysmorphisms, platelet abnormalities, neurologic complications, immune system abnormalities including T and B cell defects. We report an infant with JS found to have low TRECs on NBS and review the immune phenotypes of our cohort of 7 patients with JS.

Methods: A retrospective chart review of all patients with JS seen by the Allergy Immunology service at a large tertiary referral center from 12/1/14-12/1/19 was performed in accordance with IRB standards.

Result: The index patient had two newborn screens 24 hours after birth and two weeks later in accordance with Texas state law. The first NBS resulted normal TRECS while the second NBS had low TRECs. A third NBS was done per protocol and again showed low TRECs. Subsequently, lymphocyte subsets at 2 months of age showed severe T-cell lymphopenia: CD3 575 cells/dL (61%), CD4 351 cells/dL (21%), CD8 208 cells/dL, and low recent thymic emigrants (CD4+CD45RA+CCR7+CD31+) 42 cells/dL (12%) with normal lymphocyte mitogen proliferation. A chromosomal microarray (CMA) revealed a 11q deletion known to cause JS.

Over the five year study period we evaluated seven patients with JS referred to our center. The majority of patients (85%) presented to clinic with history of recurrent infections including recurrent pneumonia, sinusitis, otitis media, skin abscesses and warts. T-cell lymphopenia was found in 3 of 7 (43%), 2/7 (29%) had abnormal lymphocyte proliferation (mitogens and antigens) and 2 met criteria for PJP prophylaxis. In addition, 4/7 (57%) had antibody deficiency requiring IgG replacement therapy. Of the 7 cases reviewed, only 2 patients were born during the period of time that Texas was performing the NBS.

Conclusion: Jacobsen Syndrome can present with a spectrum of immune defects most notably T cell lymphopenia and antibody deficiency. These patients can present at birth with low TRECs. This cohort analysis highlights the importance of considering chromosomal genetic syndromes with features of primary immunodeficiency in evaluating patients with low TRECs. Further evaluation of larger cohorts gathered from neurology or genetics clinics at multiple centers would be helpful for future study in identifying those who need close immunology care.

(229) Submission ID#812579

The Key roles of 'IL-3' and 'Supporting Cells' for In vitro Generation of Mast Cells and Dendritic Cells from Human Bone Marrow

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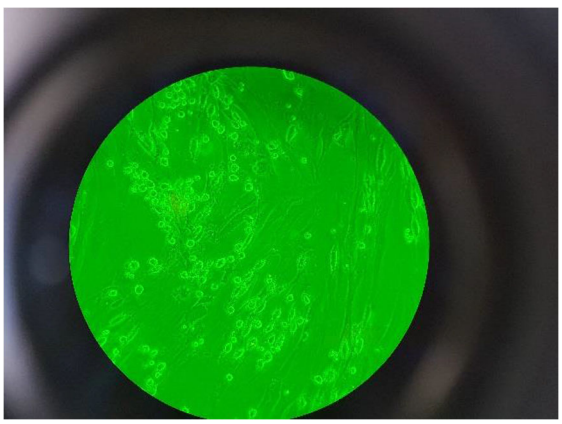
Abstract/Case Report Text

Introduction: Recognized as sentinels of the immune system, Mast Cells (MCs) and Dendritic Cells (DCs) are both derived from hematopoietic/progenitor stem cells in bone marrow (BM). The crosstalk and direct contact between these cells have been well documented and play an important role in modulating immune response. We showed that presence/absence of IL-3 directs cell fate; whether progenitor cells will be differentiated into MCs or DCs. We also report an easy method in which in vitro generation of DCs is possible without external induction of GM-CSF or IL-4.

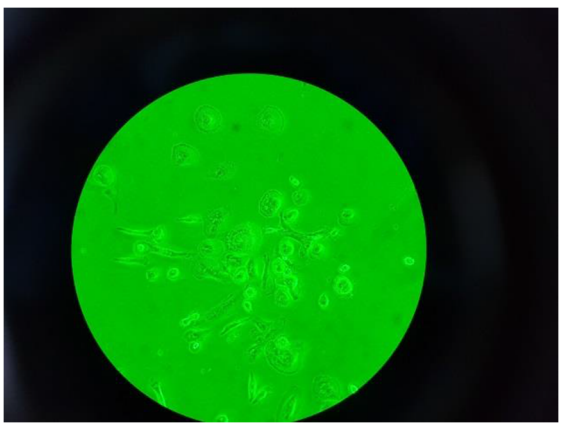
Material-Methods: To produce MCs and DCs in vitro, briefly BM samples were collected from patients with Idiopathic Thrombocytopenic Purpura., BM mononuclear cells (MNCs) were seperated by Ficoll gradient, and seeded in plates with IMDM medium containing FBS 2%, Pen/Strep, and a little amount of methocult, and incubated in 37oC, 5% CO2 (Day 0).

The treatments on the following days were as follows: Day 4, IMDM (FBS 1%) + SCF (100ng/ml) + IL-6 (50ng/ml); Day 9, IMDM (FBS 2%) + SCF (100ng/ml) + IL-6 (50ng/ml) + IL-3 (1ng/ml). On Day 18, 3 groups were formed: Group I: IMDM (FBS 2%) + SCF (100ng/ml) + IL-6 (50ng/ml) + IL-3 (30ng/ml), Group II: IMDM (FBS 2%) + SCF (100ng/ml) + IL-6 (50ng/ml), Group III: DMEM + FBS 10%. The cultures were evaluated every 2 days under an inverted microscope. Verification of MCs was performed by toluidin blue and tryptase-immunoflorescence staining. Macrophages were verified by CD-68 immunoflorescence staining. Dendritic cells of different stages of

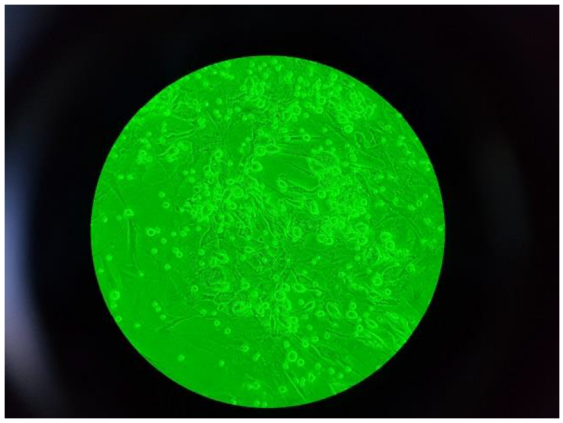
maturation were easily recognized microscopically, and evaluated with their typical appearence and elongation of dendrites.
Results: Adherent cells such as fibroblasts, endothelial and mesenchymal stem cells have grown up and adhered to the plate, thus providing an attachment site for MCs and serving as a nest for DCs to grow and proliferate. MCs' attachment to the cells at the bottom has provided medium exchange available without changing culture dishes. As the adherent cells released cytokines, growth factors, and other inflammatory mediators, they provided a natural environment for development of MCs and DCs. On day 18, when MCs formed and proliferated well, we seperated wells into 3 groups. After 1 week following our treatment of IL-3 (30ng/ml) to group I (on day 25), Group I showed proliferating dendritic cells. Group II continued to generate MCs with a low percentage of macrophages (Figures a-c) and group III consisted of mesenchymal stem cells (with only few MCs survived in DMEM) . On day 31, after a very short duration of trypsin treatment, MCs easily detached, and continued to grow and proliferate in suspension culture for about 3-5 weeks more.
Discussion: Here we report two modified methods to obtain both MCs and DCs. We determined that IL-3 dosage, and duration of treatment were the critical stimulant factors deciding the fate of the progenitor cells to differentiate into a mast cell or a dendritic cell. We also achieved generation of DCs without external induction of GM-CSF and IL-4, which are usual in vitro inducers for DC formation.



Day 16: Immature mast cells are visible (x200).



Day 25: (Group 1): 1 week after the treatment of IL-3 (30 ng/ml) in addition to SCF and IL-6, dendritic cells have formed. Dendritic cells in different stages are seen. (x400)



Day 25 (Group II): Only SCF and IL-6 were treated. Mast cells - immature and mature- have formed (x100)

(230) Submission ID#812584

Twin Infant with Prenatally Diagnosed Compound Heterozygous ADA Gene Mutations

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Abstract/Case Report Text

Background: Adenosine deaminase-1 (ADA) deficiency classically presents as severe combined immunodeficiency in early infancy, but delayed, late-onset and partial phenotypes have been reported. Here, we describe a prenatally diagnosed infant with a unique compound heterozygous genotype presenting with a normal immunophenotype after birth.
Case Description: The patient is a dichorionic-diamniotic twin-B girl, born at 35 weeks to healthy parents that are known carriers of variants in the ADA gene [mother-c.466C>T (p.R156C); father-c.452C>A (p.L152M)]. Mutation specific analysis after an amniocentesis showed that one twin was a carrier (L152M) and the other was a compound heterozygote (R156C and L152M). After birth, twin A had a mildly low erythrocyte ADA level (12.8 nmol/h/mg; normal range 63+41) with normal metabolites and a normal immunophenotype, similar to both parents. Twin B showed a normal absolute lymphocyte count and mitogen proliferation, normal T lymphocyte subsets, mildly low B and NK cells with 85% naïve T cells and a normal TREC assay. Erythrocyte ADA levels were absent in peripheral blood, with mildly elevated metabolites [dAXP=0.041 μmol/ml RBC (normal < 0.002) and %AXP=0.9 (normal < 0.2)]. Weekly recombinant ADA enzyme replacement therapy (ERT) was started at 1 week of life with subsequent normalization of the metabolites by week 14. Absolute lymphocyte, T cell subsets were normal at birth but continued to rise slightly above normal range after starting ERT. B and NK cell counts were mildly low at birth but normalized by week 3. Genetic testing confirmed the prenatal genotypes in the twin girls. The patient is now 11 months old and doing well with no history of infections. Her twin was not an HLA match and family is currently awaiting gene therapy approval.
Discussion: ADA deficient patients show substantial clinical and metabolic heterogeneity that tends to correlate with the genotype but phenotypic discordance occurs even within the same genotype. We describe an infant with prenatally diagnosed compound heterozygous mutations in the ADA gene (Grade-I: R156C and Grade-II: L152M). ADA alleles are graded from 0-IV

with increasing ADA expression and decreasing severity respectively. There are reports of children with grade I/III allele combinations with delayed, late and partial phenotypes. Two siblings have been reported with L152M allele (grade III) in combination with a different grade I allele (R235Q), presenting with combined immunodeficiency at 1 and 13 months. The specific allele combination from our patient has not been previously reported, however, we expected that the Grade-I allele likely would be more deleterious than the Grade-III allele. In our case, predicting a future phenotype remains a challenge, creating a dilemma regarding management strategies. However, with only mild metabolite elevations in our patient after birth, we may speculate whether the prenatal diagnosis with early ERT precluded the development of a full immunophenotype and it remains to be seen whether non-immune sequeli may be prevented.
Conclusion: Children with compound heterozygous mutations in the ADA gene can pose diagnostic and therapeutic challenges, especially due to the associated metabolic and clinical phenotypic variability. Early recognition and treatment may potentially alter long-term morbidity and mortality.

(231) Submission ID#812585

Jakinibs: A Bridge to Immune Reconstitution in Gain-Of-Function Signal Transducer and Activator of Transcription 1 (STAT1) Mutation

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Abstract/Case Report Text
Background: Autosomal dominant gain-of-function (GOF) mutations in STAT1 cause a syndrome of infection susceptibility, autoimmunity, and immune dysregulation. A subset of patients develop immune dysregulation-polyendocrinopathy-enteropathy-X-linked syndrome (IPEX)-like phenotype. Immunodeficiency is often combined with impairment of the humoral and cellular compartments.
Hematopoietic cell transplant (HCT) can resolve disease-related manifestations in STAT1-GOF, but overall survival is poor and there is a high rate of secondary graft loss in transplanted patients.
Jakinibs are a class of medications that block cytokine-induced JAK/STAT activation. Ruxolitinib preferentially inhibits JAK1 and JAK2 and has been used as precision-directed therapy for treatment of STAT1-GOF related manifestations with success in stabilizing and in some cases reversing organ-specific manifestations. The utility and safety of jakinibs for long term treatment of STAT1-GOF and in the prevention of disease-related manifestations is not known. As such, HCT is often pursued for patients once disease-related manifestations are controlled with jakinibs.
We present a patient with STAT1-GOF mutation with gradual secondary graft loss following HCT 10 years ago, that has had continued disease progression despite chronic ruxolitinib treatment.
Case Presentation
This is a 14 years-old male diagnosed with a de novo heterozygous STAT1 mutation (c.983A>G/A) at age 9, 5 years following HCT for IPEX-like disease. He has been treated with ruxolitinib for the last three years. This patient initially presented at 5 months of age with wasting enteropathy, failure to thrive, early-onset type 1 diabetes and hypothyroidism. He had frequent upper respiratory infections during childhood including Mycobacterium fortuitum mediastinal lymphadenitis. At 4 years he underwent 10/10 matched, unrelated bone marrow transplant following reduced-intensity conditioning. Mixed donor chimerism was present in the first 100 days following HCT, and he continued to have a slow progressive decline of donor chimerism with full graft loss (0% whole blood donor chimerism) by age 13. At age 11, enteropathy returned leading to cachexia and TPN dependence. Concurrently, he had recurrent upper respiratory tract infections, lymphopenia, and hypogammaglobulinemia. Imaging showed bronchiectasis and lung function was consistent with obstructive lung disease (FEV1:1.88 L FVC:1.96 L DLCO:13.9 ml/min/mmHg). Initiation of ruxolitinib at age 11 resolved his enteropathy with discontinuation of TPN and >25-pound weight gain. Enteropathy has not returned. Pulmonary clearance measures have also been employed. DLCO initially improved (DLCO: 19.11 ml/min/mmHg) but obstructive lung pattern continued (FEV1: 1.91 L FVC: 2.08 L). After initial improvement, DLCO began to decline. Over the last 2 years and despite treatment with ruxolitinib, lung function has deteriorated with worsened FEV1 (1.64 L), FVC (2.03L), and DLCO (17.91 ml/min/mmHg). With this progressive decline, the family is now pursuing second HCT.
Discussion
Jakinibs apply precision-directed therapy for immune dysregulatory features of STAT1-GOF. Their use leads to substantial disease control and clinical improvement but does not prevent disease progression. Jakinibs should be used as a bridge to definitive therapy with HCT in patients with STAT1-GOF mutation.

(232) Submission ID#812588

Predicting Infections Among Children With Congenital Heart Disease After Thymectomy

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Abstract/Case Report Text
Background: The thymus is the primary T-cell maturation site where T-cell proliferation, T-cell receptor rearrangement, thymocyte differentiation and maturation occur. Thymectomies are commonly performed during cardiac surgery, altering the architecture and microenvironment needed for T-cell development. Prior studies have revealed the immune impact of thymectomy, showing T-cell lymphopenia, decreased proportion of naïve T-cells, and reduced diversity of the T-cell receptor repertoire. A recent large registry study showed a higher risk of infections (HR 1.42, 95% C.I 1.33-1.52) in children with thymectomy as compared to surgery controls, in addition to demonstrating differences in the risk of cancers, autoimmunity and atopy. Limited small studies have described some risk factors for altered immune consequences; however, specific predictors of infections among children with congenital heart disease (CHD) undergoing thymectomies have not been systematically assessed. Among children with CHD and thymectomy, we sought to characterize children with and without reported infections within 4 years post-thymectomy and identify predictors of bacterial and viral infections.
Methods: Using a retrospective chart review (Institutional IRB approved) from 7/2/2013 and 3/31/2017, we identified children with CHD that underwent thymectomy and excluded any known conditions associated with immunodeficiency and those with less than 6-month follow-up post-thymectomy. First absolute

lymphocyte count (ALC) after thymectomy was stratified using a cutoff at 50% of the lower limit of age-adjusted normal values (ALC value < 50% vs ALC value >50% of the lower limit of age-adjusted normal levels). We sought to assess predictors of reported bacterial (positive blood, cerebrospinal fluid, respiratory cultures and chest-X-Ray confirmed pneumonia) and viral infections (positive viral PCR tests) within 4 years post-thymectomy.

Results: We identified 128 children with CHD who had thymectomies, of which, 65% (84/128) were male. The median age at thymectomy was 6 months (interquartile range 2 months-2.2 years); 51% (66/128) underwent a complete thymectomy; and 3% (4/128) developed a chylothorax within 1 week post-thymectomy. A substantial proportion of children had an ALC below 50% of the lower limit of age-adjusted normal levels after thymectomy (2% [3/127] pre-thymectomy vs 65% [82/127] post-thymectomy). Among children with CHD post-thymectomy, 51% (65/128) and 45% (57/128) reported bacterial and viral infections within 4 years, respectively. Children with post-thymectomy ALC values below 50% of the lower limit of age-adjusted normal levels had higher odds of reported bacterial (OR 3.44, 95% C.I 1.37-8.64, p=0.008) and viral (OR 5.86, 95% C.I 2.02-16.96, p=0.001) infections post-thymectomy as compared to those with an ALC greater than 50% of the lower limit of age-adjusted normal levels (multivariate logistic regression). There was no association with the type of thymectomy (partial vs complete), age at thymectomy, weight at thymectomy, sex or prematurity.

Conclusions: Among children with congenital heart disease with no known immunodeficiency undergoing thymectomy, ALC below 50% of age-adjusted normal levels post-thymectomy may be associated with higher odds of bacterial and viral infections. A retrospective study design with a small sample size poses several limitations; however, this study suggests that post-thymectomy absolute lymphocyte values may be a potentially useful marker to identify higher risk patients in this population.

(233) Submission ID#812589

Telomere Length: A Novel Biomarker To Predict The Presence Of Interstitial Lung Disease In PID

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Abstract/Case Report Text

Background: Interstitial Lung L Disease (ILD) is commonly encountered as a complication of many PIDs, esp. CVID in the setting of GLILD associated with CVID. Currently, there are no clear guidelines on the assessment and monitoring of interstitial lung disease in PID patients. Radiological assessments esp. in the CT chest is commonly performed, but has associated radiation exposure and pulmonary function testing, at times, maybe insensitive to small changes in lung pathophysiology.

Many PIDs may have overlapping features with Short telomere syndromes (STS) a, which are accelerated aging syndromes affecting hematopoietic, pulmonary, hepatobiliary and/or immunological systems, unified by a high cell turnover in these organs. Clinical assessment of age-appropriate telomere length (TL) is performed using flow cytometry & fluorescence in-situ hybridization (flowFISH).

Methods: We retrospectively analyzed telomere lengths in lymphocytes and granulocytes using the flow cytometry and FISH method .FlowFISH testing was done at reference laboratories in Johns Hopkins University (JHU, USA).Approval was obtained from Mayo's Institutional review board. Data abstraction and analysis was done using the software JMP.

Results: 24 patients were included in our analysis with 13 females (54%) and 11 males (45%).The median lymphocyte count of our cohort was 0.98 (0.51-1.78).The telomere length was strongly associated with the presence of lung disease (p=0.02*) and the presence of interstitial lung disease closely paralleled the changes in telomere length (delta- as compared to age adjusted normal percentiles lengths). Shorter lymphocytic telomere length was associated with more severe reduction on total Lung capacity (TLC; P=0.006*).

Conclusion: Shorter Lymphocytic telomere length served as a reliable biomarker for interstitial lung disease in PID patients. This may open up newer avenues for assessment of aging pathways in PID and may offer the option of using senolytic therapies in PIDs.

(234) Submission ID#812591

T-B+NK+ SCID Due to A Mutation in IL2RG

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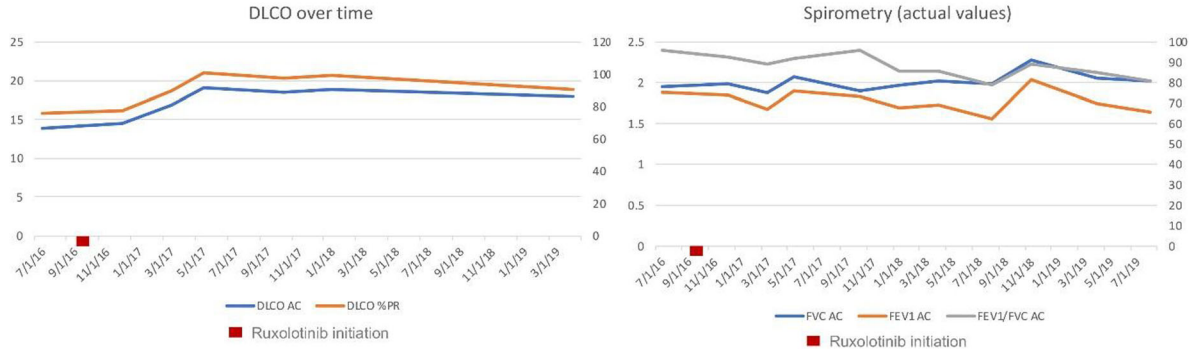
Abstract/Case Report Text

Image 1 Diffusing Capacity of the Lungs for Carbon Monoxide (DLCO) and Spirometry



Mutations in the IL-2 receptor common gamma chain gene (IL2RG) result in X-linked severe combined immunodeficiency (SCID). The common gamma chain is shared by IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 receptors. X-linked SCID typically presents with low or absent T and NK cells and normal or elevated numbers of B cells. We report a case of X-linked SCID with elevated B and NK cell numbers (T-B+NK+).

The male patient had an abnormal newborn screen for SCID in North Carolina. Lymphocyte enumeration performed at 12 days of life showed 8 CD3+ cells/mm3, 370 B cells/mm3, and 997 NK cells/mm3. He had no naïve T cells. Repeat lymphocyte enumeration two weeks later showed that the CD3+ count had increased to 389/mm3. Only 1.5% (6 cells/mm3) were CD45RA+ naïve T cells. He continued to have elevated B cell and NK cell numbers. Chimerism studies revealed the presence of 6% female cells in mitogen-stimulated PBMC by fluorescence in situ hybridization, indicating the presence of transplacentally transferred maternal cells. Lymphocyte proliferation responses to PHA and ConA mitogen stimulation were very low (less than 10% of normal). Immunoglobulin levels were IgG 921mg/dL, IgM 18mg/dL, and undetectable IgA and IgE.

Genetic studies revealed a missense mutation in IL2RG, c.467C>T, resulting in an amino acid substitution (p.Ala156Val) in the extracellular domain. Family testing showed that the patient's mother was a carrier for this variant. The father and the two healthy older brothers did not have this variant. Of note, the family history was significant for lateral maternal male early deaths.

At 7 weeks of age, the patient received an unfractionated bone marrow transplant from his HLA-identical brother without conditioning or GVHD prophylaxis. At the time of this report's submission, he is 4 weeks post-transplantation and has had successful engraftment (whole blood-CD3+ fraction was composed of >95% donor cells). He also now has normal T cell proliferation in response to mitogens and normal levels of all immunoglobulins.

Genetic defects that cause primary immunodeficiency can have variable phenotypic presentations. The patient's phenotype was atypical in that he had elevated NK cell numbers. To further evaluate these cells, we checked for STAT4 phosphorylation following IL-12 stimulation of patient NK cells. The NK cells demonstrated adequate STAT4 phosphorylation, indicating they may be functional.

Kumaki et al (Blood, 1999) reported the Ala156Val mutation in the IL2RG gene in a patient presenting with no T cells, normal number of B cells, and normal NK function. The group showed that T cells expressing the mutant gamma chain had impaired responses to IL-4 and IL-7. However, responses to IL-2 and IL-15 were maintained.

Mutations in the extracellular domain of IL2RG may result in preservation of certain signaling pathways, but not others. This may explain the presence of NK cells in this patient. However, we cannot rule out transplacental transfer of a small percentage of maternal NK cells.

Reference:

Kumaki S, Ishii N, Minegishi M, Tsuchiya S, Cosman D, Sugamura K, and Konno T. Functional role of interleukin-4 (IL-4) and IL-7 in the development of X-linked severe combined immunodeficiency. *Blood*. 1999; 93(2): 607-612.

(235) Submission ID#812594

The Natural History Of STAT3 GOF Syndrome – The Range Of Clinical Manifestations And Treatment Options

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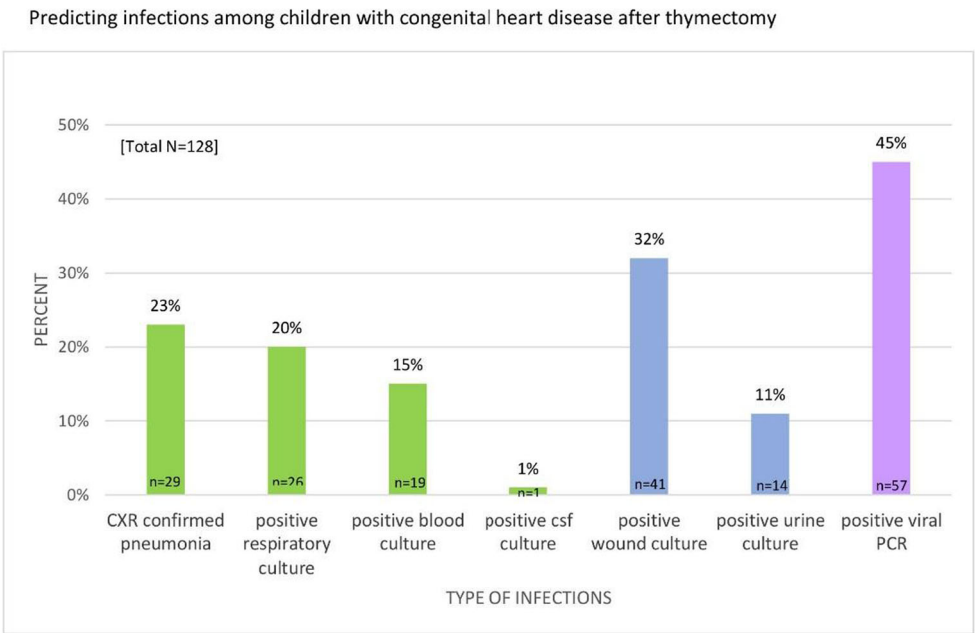

Fig 1: Percentage of types of infections seen among children with congenital heart disease within 4 years post-thymectomy.

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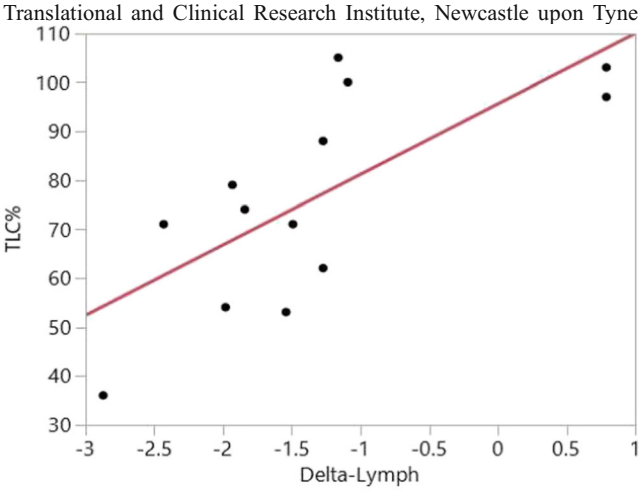

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	95.555908	7.149755	13.36	<.0001*
Delta-Lymph	14.414506	4.251122	3.39	0.0060*

Abstract/Case Report Text

Background: STAT3 gain-of-function (GOF) mutations cause a multi-system disease of early onset autoimmunity and lymphoproliferation, severe post-natal growth restriction, and recurrent and/or invasive infections. Treatment of the autoimmune and auto-inflammatory features of STAT3 GOF patients relies heavily on immunosuppression and is often challenging. The full scope of phenotypes, treatments and outcomes may be broader when analyzing a substantially larger cohort than those already reported.

Methods: We gathered and analyzed data on 144 patients from 56 centers world-wide with confirmed GOF mutations in STAT3. Retrospective chart reviews were performed in accordance with all local ethics and IRB committees to determine clinical manifestations, immunophenotype, treatment regimens, success of treatment methods, and overall survival. Funcitonal transcriptional activity was assessed by luciferase reporter assay on each individual mutation.

Results: Fifty-nine individual mutations were identified and all conferred GOF by a validated luciferase assay. There were 5 mutations in the N-terminal domain, 17 in the coiled-coil domain, 28 in the DNA binding domain, 6 in the SH2 domain, and 3 in the transactivation domain with the overwhelming majority being missense mutations. Median age at presentation was approximately 5 years; 56% of subjects are male and 44% are female. Immunodysregulatory features presented in all patients. Autoimmune cytopenias were the most common occurring in 72% of subjects (n=105), followed by lymphoproliferation in 69% (n=100) with increased frequencies of double negative (CD4-CD8-)T cells being found in 71% of of patients tested, enteropathy in 53% (n=76), endocrinopathy in 35% (n=50), interstitial lung disease in 45% (n=65), dermatitis in 41% (n=42), and inflammatory brain disease in 6.25% (n=9). Growth failure was present in 54% (n=77) with half of those patients having concurrent enteropathy. Infections were reported in 60% of the cohort to include recurrent and/or invasive viral, bacterial, opportunistic, fungal, and mycobacterial infections. Prominent abnormalities of immunophenotyping included T cell (54%) and B cell (36%) lymphopenia with reduced T cell proliferation in response to mitogens or antigens in 30% of those evaluated patients. Fifty-nine percent of the patients hypogammaglobulinemia while 40% exhibited poor specific antibody responses to recall antigens. Overall survival was 86% at data collection.Treatment of STAT3 GOF patients often included multiple agents: IVIG , chronic and pulse steroids, mTOR inhibitors, calcineurin inhibitors, rituximab, mycophenolate mofetil, alemtuzumab, tocilizumab, and jakinibs. Those started on JAK inhibition showed improvement in clinical symptoms and, to date, there are 20 STAT3 GOF patients on targeted JAK inhibition. Thus far, 18 patients have undergone bone marrow transplant with a 61% survival rate.

Discussion: STAT3 GOF mutations were first reported in 2014 to cause a heterogeneous syndrome of autoimmunity and lymphoproliferation with immunodeficiency and infection susceptibility. Earlier treatment with targeted therapy such as Jak inhibitors has led to reduced disease morbidity. We report the largest cohort of STAT3 GOF patients collected through a multi-national collaboration of the longitudinal data and natural history of STAT3 GOF disease. Understanding the heterogeneity of presentation and key features that will lead to proper diagnosis and early treatment in an effort to prevent long term disease associated sequelae.

(236) Submission ID#812602

Novel Mutation in Transcription Factor 3 (TCF3) Associated With A Unique Humoral Phenotype and Severe Thrombocytopenia Responsive To Rapamycin

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Abstract/Case Report Text

We present the case of 5 month old male with a novel heterozygous mutation in TCF3 and two previously unreported phenotypes: 1) absent circulating CD19+ B cells yet preserved immunoglobulin synthesis and vaccine responses and 2) significant thrombocytopenia that improved with immunosuppression. There is also a striking family history of two half-sisters who died during early infancy with similar clinical and lab findings and the same genetic change.

The infant boy was born at term with respiratory failure and generalized rash. At birth he had thrombocytopenia (24k/uL) and lymphopenia (465/uL). Initial absolute CD3+ T cell count was low (442/uL), yet he had normal thymic output and proliferative responses to mitogens ruling out SCID. CD19+ B cells were < 5/uL and bone marrow biopsy revealed decreased hematagones, yet he had a normal IgM level (23.1 mg/dL) elevated IgA (56 mg/dL), and elevated IgE (27 IU/ml). IgG levels were initially obscured by maternal IgG and IVIG; in turn he made positive titers to diphtheria and tetanus vaccination. The infant has maintained his own IgG production.

Rapid genome sequencing revealed a heterozygous predicted deleterious VOUS in TCF3, in the second transactivation domain (c.1138 C>T, p.Pro380Ser). The same change in TCF3 was identified in the deceased half-sisters as well as the father: all 3 infants had different mothers, suggesting autosomal dominant inheritance. The sisters had similarly severe thrombocytopenia and absent circulating B cells; their causes of death were not completely understood. The 33 year-old father has normal platelet levels, very low CD19+ B cells (35/uL), elevated IgG (1,580 mg/dL) and IgE (1,030 IU/mL), and normal levels of IgA and IgM. The father also has an elevated number of CD3+ T cells (3,455 /uL) with an increased percentage of T cells expressing HLA-DR (46%).

In the months after birth, the infant boy continued to require frequent platelet transfusions. Despite the persistent T lymphopenia, there was evidence of increased T cell activation with elevated levels of soluble IL-2R (2510 pg/ml) and increased percentage of cells expressing HLA-DR (30%) CD95 (98%), CD25 (86%), CD71 (55%), and CD69 (14%). A 10-day trial of prednisone was associated with an increase in his platelet count to >100k/ul. He was switched to rapamycin as a steroid-sparing agent, and his platelet count has remained > 200k/ul for several weeks without transfusions. Interestingly, his B cell counts also improved after the steroid trial (50/uL) and his absolute lymphocyte count is normalizing on rapamycin. A potential mechanism could be rapamycin decreasing T cell-mediated destruction of platelets or B cells. Reassessments of T cell activation markers and B cell phenotyping while on rapamycin will be done in the future.

In contrast to multiple published cases of TCF3 mutations associated with complete agammaglobulinemia and absent B cells, we present a case of an infant with absent B cells yet preserved humoral function as well as severe thrombocytopenia responsive to rapamycin. In collaboration with colleagues at NIH, studies are underway to understand whether/how the unique change in TCF3 is related to either phenotype described above.

(237) Submission ID#812603

ARPC1B Deficiency Presenting in an Adult Female with Lymphadenopathy, Cytopenias, and Polymorphic Ulcerative, Vasculitic and Epidermodysplasia Verruciformis-like Mucocutaneous Manifestations

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Abstract/Case Report Text

Background: Childhood-onset, chronic, multi-system inflammatory diseases are increasingly being characterized as monogenic inborn errors of immunity. ARPC1B deficiency is a recently described, rare combined immunodeficiency characterized by recurrent/severe infections, a variety of autoimmune manifestations and platelet defects. We describe a case of ARPC1B deficiency identified in an adult patient with recurrent ulcers/Bechet-like disease, non-malignant lymphoproliferation and intermittent microthrombocytopenia.

Patient Case: At 1 year of age, our female patient was diagnosed with Behcet disease based on a history of bloody stools at 3 months, oral ulcers at 9 months and vulvar lesions at 1 year. She underwent rheumatology evaluations for inflammatory arthritis, episcleritis, eczema, vasculitic ulcerating nodules of the trunk, perineum and extremities, and verrucae forming flat plaques similar to epidermodysplasia verruciformis without a unifying diagnosis. Other infections include otitis media, sinusitis, Pseudomonas ecthyma gangrenosum, cervical lymphadenitis, and pneumonia.

At 28 years old, the patient was referred to our Immuno-Hematology Comprehensive Program Clinic with a concern for malignancy versus a primary immune regulatory disorder (PIRD). She had a 6-month history of drenching night sweats, urticarial plaques, edema in her extremities and diffuse cervical, axillary and inguinal lymphadenopathy. Past complete blood counts showed intermittent mild microthrombocytopenia. Lymph node biopsies were negative for a neoplastic process but identified plasmacytosis, including focally increased IgA-kappa+ plasma cells. Expert review of the lymph node biopsy, and further evaluation excluded multicentric Castleman disease. Consideration was also given to autoimmunune lymphoproliferative syndrome (ALPS)-like disorders;

however, her ALPS flow cytometry panel was nondiagnostic. Her basic immune evaluation showed severe T cell lymphopenia (CD3+ 229 cells/cm, CD4+ 222 cells/cm, CD8+ 52 cells/cm) with adequate B and NK cells, normal lymphocyte proliferation to PHA and PWM, and dysgammaglobulinemia with IgG 1579 G/dL, IgA 638 G/dL, IgM 50 G/dL and IgE 592 G/dL.

Due to concern of an underlying PIRD, a primary immunodeficiency panel was sent for 207 gene analysis with negative results. However, trio clinical exome sequencing identified biallelic variants in the gene ARPC1B. One allele has a truncating, nonsense pathogenic variant in exon 8 denoted as c.898G>T, p.Glu300Ter. The other allele has a likely pathogenic variant in intron 4 denoted as c.393-2A>G, resulting in disruption of the canonical splice acceptor for exon 5. This is predicted to cause exon skipping, with an in-frame deletion of amino acids coded by exon 5.

Conclusion: This case highlights the value for evaluation for PIRDs in patients presenting with Behcet-like disease, particularly in the context of other autoimmune manifestations and/or microthrombocytopenia. It also underscores that patients with ARPC1B deficiency may present with chronic non-malignant lymphoproliferation. Moreover, this patient emphasizes the value of exhaustive genetic testing for complex immunologic phenotypes.

(238) Submission ID#812605

LIPT1 Defect Associated With Agammaglobulinemia In A Neonate

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Abstract/Case Report Text

Lipoyltransferase 1 gene defect is associated with severe mitochondrial dysfunction disrupting lipoic acid biogenesis. Clinical manifestations associated with early seizures, hypotonia, cardiomyopathy and pulmonary hypertension and encephalopathy. Early neonatal death due to sepsis and cardiovascular collapse is commonly seen.

The patient is a 36 week preemie male with congenital heart disease who developed severe intractable lactic acidosis on day of life 1 with increased excretion on organic acids of 2-methyl-2,3-dihydroxybutyric acid. A mitochondrial disorder , ECHS1 or HIBCH deficiency was suspected. At 3 mo of age the patient was admitted for apneic spells and respiratory compromise. He was found to have elevated CRP associated with Rhinovirus infection and gram-negative bacteremia. Due to the history of failure to thrive and sepsis, Immunology was consulted. Immunologic work up indicated normal B, T and NK cells with normal DHR, but showed agammaglobulinemia. The patient was started on ivig and whole exome sequencing was done. Molecular analysis showed compound heterozygote mutations in the LIPT1 gene: c.293G>A (p.Arg98Gln) and c.635T>G (pVal212Gly). Subsequent biochemical analysis also showed biochemical abnormalities consistent with LIPT1 defect. Lipoyltransferase 1 is an enzyme involved in activation of a number of enzymes requiring lipoic acid. It is involved in lipoic acid synthesis. Lipoic acid is required for the activity of pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase, and branched-chain alpha-ketoacid dehydrogenase. The literature indicates that most patients with LIPT1 defect have a severe, often fatal course. The patient is now almost 3 years old and has stable clinical course without any major infections. He certainly has significant hypotonia and developmental delay.

In conclusion, we are presenting the first case of LIPT1 gene mutations associated with agammaglobulinemia who responded well to ig supplementation therapy. Our immunologic findings in this case highlights the importance of immunodeficiency work up in challenging cases. As we see more cases LIPT1 gene mutations, we will better understand the clinical spectrum.

(239) Submission ID#812607

Hereditary Alpha Tryptasemia Presenting With Scurvy

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Abstract/Case Report Text

A now 8-year-old male was initially evaluated for concerns regarding food allergy, eczema, Food Protein-Induced Enterocolitis Syndrome, and failure to thrive. He had reactions of varying severity to multiple foods. These usually involved immediate urticaria or prolonged vomiting, diarrhea, and abdominal pain. IgE and skin prick testing was performed to suspected foods and was positive to milk, egg, pork, wheat, peanut, pecan, coconut and corn. These foods had historically caused reproducible immediate symptoms. Testing was negative to other suspected foods. He developed an oral aversion and extremely restricted diet. Symptoms of abdominal pain, hematochezia, rashes, arthralgias, headaches, fatigue, dyspnea, and palpitations increased. Urticaria and severe abdominal pain with vomiting and diarrhea continued intermittently without identifiable triggers on a restricted diet. Laboratory markers demonstrated elevated inflammatory markers, anemia, iron deficiency, vitamin B12 deficiency, and vitamin C deficiency (scurvy). Gastroenterology work up did not identify any pathology. Gastrointestinal symptoms did not respond to treatment with multiple GERD medications or oral steroids.

Baseline tryptase was elevated. Low histamine diet was initiated and repeat tryptase remained elevated. Fractionated Tryptase revealed normal mature (beta) tryptase with elevated total tryptase, negative genetics for c-KIT mutation, normal urine prostaglandins. Family members had tryptase levels drawn. One parent and sibling had elevated Tryptase levels, while the other parent's Tryptase was normal.

Hereditary alpha tryptasemia syndrome is defined by elevated blood tryptase levels and symptoms involving multiple organ symptoms. Patients with elevated tryptase levels without symptoms are defined as having Hereditary alpha tryptase trait. There is significant variability regarding which patients are symptomatic. Organ symptoms that may be involved include skin, gastrointestinal, neurologic, connective tissue, cardiac, neuropsychiatric. Severe allergic reactions such as anaphylaxis can occur. Increased blood levels of the protein tryptase are caused by extra copies of the alpha tryptase gene (TPSAB1). Treatment is usually directed at specific symptoms, antihistamines, and mast cell stabilizers. Research continues into additional treatment options.

This patient was started on Cromolyn and long-acting antihistamine. His gastrointestinal symptoms and rash/urticaria improved, and he began

tolerating a small, but increased, variety of foods. The majority of his constitutional symptoms of fatigue, arthralgias, weakness resolved as he began gaining weight, and hemoglobin, vitamin C and B12 normalized. His sibling was evaluated and noted to have food allergy, asthma, abdominal pain, GERD, and eczema. She was also started on Cromolyn and antihistamines which improved her gastrointestinal symptoms. Parent with elevated tryptase was recommended to be evaluated further with Allergist.

This is an example of a patient with elevated Tryptase and multiple organ system involvement. Some of his signs and symptoms responded to mast cell stabilizing and antihistamine medications. Patients with history of recurrent episodes of allergic reactions to foods and multiple constitutional symptoms would benefit from baseline tryptase levels. Family members should also be tested if the patient has elevated tryptase.

(240) Submission ID#812610

Neonatal Screening for SCID: Development of a TREC newborn screen in Alberta, Canada, and comparative assessment of SCID demographics before and after screening implementation

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Abstract/Case Report Text

Introduction : Population based newborn screening for severe combined immunodeficiency (SCID) has been implemented throughout the United States. Within Canada it is only available in Ontario and Nunavut (2013), the Maritimes (2016) and Alberta and the Northwest Territories (2019). Multiple studies have been published looking at the rates of SCID in the United States. The estimated rate of SCID prior to screening was 1 per 100 000 live births. Post screening implementation, on average rates of SCID were found to be closer to 1 in 60 000 live births.

Results : Development of a T cell receptor excision circles (TREC) SCID screen in Alberta involved the screening of 4000 anonymous term neonates using quantitative PCR for TRECs. The cycle threshold for the control gene, RNaseP, was set at 30.5 as 95% of our population had a cycle threshold < 30.5 (90% CI [30.4,30.6]). From those bloods spots with adequate DNA, a final TREC cut off of 40 was chosen, as it would give an accuracy of 99.6%, and fairly low false positive rate of 0.4% (95% CI [0.002, 0.006]).

Since starting a population based screen for SCID in June of 2019, we have identified 4 cases of SCID and 13 cases of low TREC not caused by SCID. To date we have detected one case of reticular dysgenesis, 2 cases of ADA SCID and one case of X- linked SCID. Other causes of lymphopenia in the neonatal period detected with abnormal TRECs include one syndrome associated with variably affected cellular immunity (CHARGE) and 12 cases of secondary lymphopenia including four cases of prematurity, three cases of diaphragmatic hernia or gastroschisis, four patients with underlying cardiac disease, and one patient with severe hydrops.

Discussion : Canada has multiple unique populations with increased risk of SCID. The estimated rate of SCID in Canada prior to implementation of a population based screen was 1.4 per 100 000 live births. The rate within Canada's First Nations, Métis and Inuit populations is 4.4 per 100 000 live births.

Prior to SCID screening, Alberta had 13 cases of SCID identified between 2005-2014 with an estimated rate of 1 per 60 000 live births. To date, our screen in Alberta has identified 4 cases of SCID with a rate of 1 per 7000 live births which is significantly higher than previously estimated. Given that early diagnosis and definitive management through bone marrow transplant or gene therapy has been shown to reduce mortality this screen will help reduce morbidity and mortality in this vulnerable population.

(241) Submission ID#812611

Chromosomal Integration of HHV-6 Masquerading as Viral Susceptibility

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Abstract/Case Report Text

Introduction: Human herpesvirus 6 (HHV-6) has the ability to integrate its genome into host telomeres. If this integration occurs in gametes, then the virus can be genetically transmitted and offspring will carry a copy of chromosomally integrated HHV-6 (ciHHV-6) in each somatic cell. This can lead to false attribution of infectious and non-infectious presentations of HHV-6, and make the diagnosis of active HHV-6 infection difficult. We present the case of a patient with meningoencephalitis attributed to HHV-6 and persistently elevated blood levels of HHV-6 by PCR concerning for primary immunodeficiency who was discovered to have ciHHV-6.

Case Description: A 7-year-old female who carried a past diagnosis of HHV-6 meningoencephalitis was seen in Immunology clinic for follow up of persistently elevated levels of HHV-6 DNA in her blood by PCR. She was born at 35 weeks and as an infant had failure to thrive (FTT), anemia and a varicella like rash after varicella immunization. At the age of 3, she received FluMist vaccine and developed a fever the following day. Over the next few days, she developed lethargy, altered mental status, headache, photophobia, seizure, papular rash and oral ulcers. She was admitted to the hospital and CSF studies were consistent with viral meningoencephalitis (270 WBC, 30L, 63M, 100 RBC) although HSV, CMV, EBV and enterovirus were negative. She was treated for presumed HSV encephalitis with 21 days of IV acyclovir and 5 days of high dose steroids. One week after discharge, she again developed papular rash on feet, headache, oral ulcers and lethargy. She was admitted and CSF studies this time showed only 3 WBC but positive HHV-6. Blood and skin swab were also positive for HHV-6. Immunology was consulted while admitted and work up for primary immunodeficiency was initiated. Her work up was normal including responses to vaccine titers, complement studies,

functional NK assay, T/B/NK panel, TLR assay, lymphocyte mitogen assay, tetanus antigen stimulation assay and whole exome sequencing. She was found to have humoral immune deficiency (low IgG and decreasing IgM) and was started and maintained on scIg replacement. She was followed over time and her blood remained PCR positive for HHV-6 with viral loads between 4-7 million copies/ml while asymptomatic. She was referred to Infectious Disease who noted that her persistently high levels of HHV-6 were concerning for ciHHV-6. Her father was tested and had a viral load of 1200 copies/ml suggesting inheritance of ciHHV-6.
Discussion: Chromosomally integrated HHV-6 should be a consideration in cases of persistently elevated HHV-6 levels. Given that any specimen obtained will contain HHV-6 DNA, this may lead to misdiagnosis of active HHV-6 infection. In return, this may result in the patient receiving unnecessary or incorrect treatment or missed diagnosis of other etiology. It is important to note that HHV-6 levels will oftentimes exceed those seen during active infection. Viral loads of greater than 1 million copies/ml are especially concerning for ciHHV-6. Therefore, cases of persistently high HHV-6 levels, especially when asymptomatic, should prompt the provider to consider ciHHV-6.

(242) Submission ID#812615

Truncating Mutations in SAMD9L Cause an Early-Onset Immune-Dysregulatory Syndrome of Neutrophilic Panniculitis, Interstitial Lung Disease and Cytopenias

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Abstract/Case Report Text
Background/Purpose: The Sterile Alpha Motif Domain Containing 9 Like protein that is encoded by SAMD9L plays a role in endosome fusion, and deletions (haploinsufficiency) of SAMD9L including loss of the chromosome 7 where SAMD9L is located (monosomy 7) have been associated with myelodysplasia in humans and mice. Missense mutations in SAMD9L were described in patients presenting with ataxia-pancytopenia syndrome. Here we describe 6 patients with de novo frameshift mutations in SAMD9L who present with early-onset systemic inflammation, variable interstitial lung disease and cytopenias.
Methods: Whole exome/genome sequencing (WES/WGS) on trios using Illumina HiSeq 2000 platform were performed. An interferon-response-gene score was assessed using a customized Nanostring assay and RNA-seq was performed in patients and healthy controls (HCs). Toll-like receptor (TLR) stimulation assays and STAT phosphorylation assay were performed in patients and HCs, PBMCs, monocytes and T cells.
Results: We identified 6 patients with 4 de novo frameshift variants in SAMD9L (c.2626delA, p.I876Lfs*15; c.2633delA, p.K878Sfs*12; c.2658_2659delTT, p.F886Lfs*11; c.2666delT, p.F889Sfs*2). All 4 variants detected were not detected in public databases (gnomAD and 1000G). Somatic reversion restricted to hematopoietic cells was observed in 1 patient. All 6 patients had disease onset between 1 and 7 days of life with generalized nodular skin rashes, fever and increased inflammatory markers (ESR and CRP). Skin biopsies from all 6 patients revealed a neutrophilic panniculitis. Four patients had developed severe interstitial lung disease (ILD) triggered by respiratory viral infections in infancy, all 4 developed pancytopenia, low B-cell count and hypogammaglobulinemia, and two of those underwent bone marrow transplant. The other 2 patients developed leukopenia, a low B cell count, hypogammaglobulinemia and recurrent pulmonary infiltrates at the age of 3 and 5 years, respectively. Additionally, brain imaging revealed basal ganglia calcifications and/or demyelinating changes in 5 out of the 6 patients. qRT-PCR of healthy control cell subsets and tissues showed that SAMD9L mRNA relative expression is high in B and NK lymphocytes,

moderate in T cells, monocytes, neutrophils, lung and muscle, and low in skin, liver, heart and kidney tissues. Analysis of each individual gene expression level by nanostring in comparison with healthy controls demonstrated significantly higher levels of the following IRGs: DDX60, EPSTI1, GBP1, IFI6, ISG15, LY6E, OAS1, OAS2, OAS3, RSAD2, RTP4 and SOCS1. Whole blood RNA-seq was performed in 3 patients and pathway analysis with the differentially upregulated genes demonstrated an enrichment of intraluminal vesicle formation and negative regulation of apoptotic signaling pathways. Stimulation of PBMCs with the TLR ligands poly I:C, ODN, and LPS induced a 200-fold increase in IFI27 and a 30-fold increase in IFNA1 and IFNB1 transcription compared to baseline. One patient had constitutive upregulation of STAT1 and STAT6 in monocytes and of STAT1 and STAT3 in T cells.
Conclusion: We describe a novel immunedysregulatory disease caused by de novo truncating variants in SAMD9L that presents similar to CANDLE with neutrophilic pannicultis and points to an important role of SAMD9L on regulation of adaptive and innate immune responses.
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Ataxia Telangiectasia with Recurrent urinary tract infections and Sepsis

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Abstract/Case Report Text
Introduction: Ataxia Telangiectasia (AT) is caused by a defect in the ATM gene which is responsible for repair of damaged DNA. It is a rare, devastating neurodegenerative disease that results in ataxia and telangiectasias, particularly of the sclera and skin. Those with AT are at increased risk for immunodeficiency and cancer. The immunodeficiency is variable and may result in deficiency in humoral and cellular immunity in some patients. Here we present a patient with Ataxia Telangiectasia and hypogammaglobulinemia on immunoglobulin therapy who developed recurrent urinary tract infections (UTI) and sepsis.
Case Presentation: The patient is a 23-year-old female with Ataxia Telangiectasia and hypogammaglobulinemia who presented with three episodes of UTI, one of which resulted in prolonged hospitalization due to sepsis and acute kidney injury (AKI). She presented with 4 days of flank and back pain and was hospitalized for 3 days for E coli UTI. She improved on IV antibiotics and was discharged home to complete treatment with oral Ciprofloxacin. Due to persistent emesis, she was readmitted 2 weeks later with urosepsis and AKI with a creatinine of 2.06 mg/dL, over 5 times her baseline creatinine. After additional antibiotics and IV fluids, she improved clinically, renal function normalized and she was discharged home. Renal ultrasound was unremarkable with no anatomical abnormalities.
She was relatively healthy prior to this with only one episode of bacterial pneumonia in 2006. She receives weekly subcutaneous immunoglobulin therapy dosed at 120 mg/kg with normal IgG levels (1023, 931, 1078 mg/dL). At baseline, she had high IgM (550 mg/dL) and low IgA (< 4 mg/dL) levels, as well as decreased T cells but normal NK and B cells (489 cells/uL CD3+, 329 cells/uL CD4+, 107 cells/uL CD8+, 211 cells/uL CD16+CD56+, and 357 cells/uL CD19+). She has hyperglycemia (on Metformin), hypertriglyceridemia (on Atorvastatin) and hypertension (on Losartan). She is thin and wheelchair bound with bilateral telangiectasias to the sclera, neck, and chest. She has occasional eye bleeding and epistaxis, presumably from her telangiectasias. She has good hygiene and good adherence to medications. She voids voluntarily, has no indwelling urinary catheter and is not sexually active.
Discussion: Patients with Ataxia Telangiectasia may have frequent viral and bacterial infections, most frequently upper and lower respiratory tract infections, as well as wart and skin infections. Based on our review, this is the first reported case of Ataxia Telangiectasia with hypogammaglobulinemia on immunoglobulin therapy with recurrent UTIs complicated by urosepsis and AKI. Despite adequate IgG levels on immunoglobulin therapy, our patient continued with recurrent UTIs. It is uncertain whether her non-ambulatory status, hyperglycemia or related immunodeficiency are the causes for her increased susceptibility to UTIs. The literature reports patients with AT and bladder wall telangiectasias can result in significant hematuria, and perhaps this may be a source of entry for bacteria and consequent development of UTI. This suggests that patients with Ataxia Telangiectasia and recurrent UTIs may benefit from renal ultrasound and possible cystoscopy to better visualize telangiectasias. We recommend consideration of workup for recurrent UTIs in patients with Ataxia Telangiectasia.

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The Fate of Early Polyreactive B Cells in Partial RAG deficiency

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Abstract/Case Report Text
Objectives: Patients with partial Rag deficiency frequently present with humoral autoimmunity suggesting breach in tolerance mechanisms and subsequent expansion of autoreactive B cell clones. Here we aim to trace polyreactive B cells and their descendants at B cell developmental stages through our in-house bioinformatic pipeline, ImmChainTracer (ICT).
Methods: The B cell receptor (BCR) was expressed as monoclonal antibodies from single sorted mature naive B cells (n=30-50 per donor) from patients with hypomorphic RAG deficiency and healthy donors. xThe recombinant monoclonal antibodies were screened for polyreactivity (dsDNA, insulin, LPS and IFNα) by ELISA. In parallel, IgH repertoires were deep sequenced from sorted mature naïve, activated naïve and memory B-cell compartments. Our in-house assembled bioinformatic pipeline called ImmChainTracer (ICT) was applied to track down the descendants of cloned autoreactive IgH sequences in repertoires of subsets above.
Results: IgH sequences (n=125 including 45 polyreactive, 80 non-polyreactive clones) from mature naive B cell from six patients with partial RAG deficiency and 3 healthy donors (n=91 including 7 polyreactive, 84 non-polyreactive) were analyzed with our novel in-house bioinformatic approach to track lineage fate in repertoire at specific developmental stages. Interestingly, 28.8% of the patients' sequences and their descendants were identified in their mature naive, activated naive or memory B cell repertoires, while none of the analyzed healthy donor clones were found at later subsets. Furthermore, genealogical analyses of related clones revealed lineage expansion and progressive positive antigen selection of the autoreactive clones in the patients.
Conclusions Our findings demonstrate that peripheral tolerance checkpoint is broken in hypomorphic RAG patients. Our novel method enables tracing the fate of autoreactive naive B cells in the effector repertoires. We have shown that impaired B cell tolerance allows the expansion and

persistence of autoreactive, potentially harmful B cell clones. These clones reach the effector B cell compartments (memory B and plasmacell niches) resulting in the generation of autoantibodies characteristic to hypomorphic RAG deficiency.

(245) Submission ID#812628

Reduced Toxicity Allogeneic Hct With A Busulfan, Fludarabine Regimen: A Promising Approach For Non-CGD Primary Immune Deficiencies Requiring Myeloablation?

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Abstract/Case Report Text

INTRODUCTION A reduced toxicity busulfan, fludarabine regimen with alemtuzumab or anti-thymocyte globulin (Gungor et al.) was efficacious in patients undergoing allogeneic HCT for CGD. We report our experience with a similar approach for patients with non-CGD primary immune deficiencies needing a reduced toxicity myeloablative approach.

METHODS We retrospectively reviewed records of consecutive patients who underwent allogeneic HCT for primary immune deficiencies with a preparative regimen containing busulfan, fludarabine and alemtuzumab or anti-thymocyte globulin(ATG), at three transplant centers between 2015-2018. Busulfan was given either every 6 hours over 4 days with target AUC of 875 to 1025 μMol/min (based on q6 hr. dosing) or twice daily over 4 days with target AUC of 1800 to 2000 μMol/min (based on q12 hr. dosing) or once daily over 4 days with a target AUC of 3600-4400 μMol/min (based on q24 hr. dosing). Fludarabine 150 mg/m2 to 180 mg/m2 was given divided over 4-6 days. Serotherapy included alemtuzumab 0.3 – 1.0 mg/kg or ATG 7.5 mg/kg given divided over 3 days. GVHD prophylaxis consisted of cyclosporine and mycophenolate mofetil.

RESULTS Forty patients (WAS=12, HLH=10, CD40L deficiency=7, IPEX/VEOIBD=4, SCN=2, IFNGR1 def./CID/X-SCID/MSN1/LAD=1) received busulfan, fludarabine and alemtuzumab or ATG for allogeneic HCT (first HCT in 31 patients and second HCT in the 9 patients with HLH). Median age was 2.0 years (range, 0.25 years – 19.8 years). Patients received a graft from an HLA-matched related (n=11), unrelated (n=27), or single allele mismatched related or unrelated donor (n=2). All except one patient engrafted at a median of 13 days (range,11-34 days). One patient developed veno-occlusive disease and two patients developed diffuse alveolar hemorrhage. Notably, it was the second transplant for all 3 patients. Eight patients (20%) developed grade 2-3 acute GVHD and 2 patients (5%) developed chronic GVHD. One patient developed primary graft failure and two patients secondary graft failure. Nineteen patients (48%) maintained full donor (>95%) chimerism following allogeneic HCT. Twenty patients (50%) developed mixed chimerism, predominantly in the T-cell lineage, but T-cell donor chimerism progressively increased post-HCT. At 1-year post-transplant, 15 of 20 patients (75%) with mixed chimerism had donor myeloid chimerism >90% and T-cell chimerism >75%. Two patients underwent a second transplant for graft failure. There were 6 deaths in the cohort. Overall survival was 85%(34 of 40) and event free survival was 80%(32 of 40) at 1 year.

CONCLUSION Our experience suggests that a reduced toxicity busulfan, fludarabine regimen with alemtuzumab or ATG as serotherapy offers a promising approach with low toxicity, durable myeloid engraftment, low incidence of grade 2-4 GVHD and excellent survival and can be considered for a variety of primary immune deficiencies where myeloablative HCT is desired.

(246) Submission ID#812634

New Treatment Approach To Anti-NMDA Receptor Encephalitis Associated With Catatonia

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Abstract/Case Report Text

This is an 18-year-old patient who has the diagnoses of anti-NMDA receptor encephalitis with associated catatonia. The patient has history of a pineal gland germinoma diagnosed in August 2018 after 8 months of double vision. However, after several months, the patient developed difficulty in sleeping, anxiety and nightmares. The patient presented to Emergency Department in February 2019 with personality changes. He was diagnosed with NMDA encephalitis based on the clinical findings as well as presence of elevated serum and CSF anti-NMDA antibodies. The patient was initially treated with high dose systemic steroids with poor response. Due to the worsening of his clinical condition, he was started on plasmapheresis, but had poor response to this therapy as well. These treatments are known standard treatment options for this condition. During his hospital stay, different therapies were discussed. Cyclophosphamide was one of the treatment options, but because of the side effect profile and severe toxicity, we recommended different treatment modality. We started the patient on rituximab as well as sirolimus therapy to suppress both T and B-cell responses. After receiving two doses of rituximab in addition to daily sirolimus, the patient showed improvement of his symptoms. And declining NMDA receptor antibody titers.

This treatment plan was chosen for autoantibody mediated encephalomyelitis due to the fact that rituximab has inhibitory effect on naive B cells, but not on the proliferation of memory B cells and Sirolimus has profoundly inhibiting role on memory B cells as well as a T-cell responses.

Combination of sirolimus and rituximab therapy controlled his autoantibody production which was an important goal for his autoimmune condition.
We present a new treatment approach for Anti-NMDA receptor encephalitis. Rituximab was tried on these cases before but the combination of sirolimus and rituximab therapy was never given before. We now recommended that on refractory cases of Anti-NMDA receptor encephalitis, combination of Rituximab and Sirolimus therapy can be tried.

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Mosaic Variants In Immune Function Genes Identified Through Exome Sequencing

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Abstract/Case Report Text
A mosaic gene variant is one which is present in some, but not all, cells within an individual. They are increasingly associated with a number of diseases, including a number of primary immunodeficiency disease (PID). Here we systematically analyzed mosaic variants in known or putative immunodeficiency genes from exome sequencing data from 998 individuals. Lofreq was used to identify mosaic variants with a variant allele fraction (VAF) of 0.05-0.30 (0.50 is the VAF for a non-mosaic, heterozygous variant). We removed variants from extreme read-depth (> 2 standard deviations), unmappable, repeat-rich, and duplicated genomic regions. The average number of detected variants per MB was 3.2 and the total number of genomic locations with variants was 65,446. Mosaic variants were underrepresented in exonic regions, suggesting that coding variants may be deleterious. More mosaic variants were detected in saliva exomes compared to peripheral blood exomes (p-value < 1 x 10-5), suggesting tissue-specific mosaic variants in buccal epithelial cells and white blood cells of saliva. To understand the clinical relevance of these findings, the variants were further filtered to include only nonsynonymous variants found in fewer than 1% of samples, leaving a total of 6,808 variant locations. Of the remaining variant locations, 40 had a pathogenic assertion in the human gene mutation database, and 140 were in International Union of Immunological Societies PID genes. Further variant interpretations and clinical correlations are underway. These data suggest that mosaic variants in PID genes are common, vary by location of collection, and may have clinical diagnostic relevance.

(248) Submission ID#812640

A rare case of ARPC1B deficiency causing hemorrhagic gastroenteritis, failure to thrive, and thrombocytopenia

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Abstract/Case Report Text
Introduction/Background: The ARPC1B (actin related protein 2/3 complex subunit 1B) gene is a protein coding gene prominently expressed in blood cells and is necessary for the assembly and maintenance of the human actin-related protein 2/3 complex (Arp2/3). Actin polymerization plays a central role in many immune functions including proliferation and differentiation of immune cells, migration, intercellular and intracellular signaling and activation of both innate and adaptive immune responses. Defects in the actin cytoskeleton affect hematopoietic cells in the bone marrow and the immune response giving rise to a distinct primary immune deficiency, which is phenotypically similar to Wiskott-Aldrich Syndrome (WAS). ARPC1B deficiency clinically presents as a severe multisystem disease which includes platelet abnormalities, recurrent infections, failure to thrive, inflammatory changes in the intestine, eczema, cutaneous vasculitis, eosinophilia, and elevated inflammatory markers. ARPC1B deficiency is rare and has only recently been described in the literature. Here we present a clinical case of a patient found to have a pathogenic ARPC1B mutation via whole exome sequencing.
Case report: Here we describe a 15 month old Somalian boy who presented to the immunology team at 9 months of age with hematemesis, hematochezia, melena, failure to thrive, atopic dermatitis, hypothyroidism, autoimmune thrombocytopenia and recurrent infections concerning for a primary immune deficiency. Family history was notable for parental consanguinity and an older sibling with a similar presentation of hemorrhagic gastroenteritis who died in Kenya around 3 months of age due to complications of his symptoms. The initial primary immunodeficiency evaluation revealed normal inflammatory makers, normal IgG and protective vaccine (Prevnar and tetanus) response. IgA and IgE were elevated at 364 mg/dl and 99.6 KU/L respectively. Flow cytometry was remarkable for T cell lymphopenia (CD3 1541, CD4 1163, and CD8 363) with reduced naïve CD4 and CD8 T cells. B and NK cell count were normal. ALPS panel and WAS protein expression was unremarkable. Whole exome sequencing was performed and revealed homozygotic mutation of ARPC1B c.392T>C, IVS4+2T>C which was predicted to be a pathological variant. Subsequently, DHR flow cytometry with fMLP showed significant increase of DHP fluorescent (MFI 9.7 when compared to control MFI 3.7) consistent with findings from other ARPC1B deficiency patients. At the time of the most recent clinic visit, the patient has remained

stable with no interim infections and is doing well on thyroid hormone replacement. The current plan is for the patient to undergo a stem cell transplant for the ARPC1B deficiency as he is at high risk for recurrent infections and severe disease. Although this gene mutation is rare, review of the current literature describes patients with this condition that have undergone stem cell transplant and have done well. At this time, this seems to be the best option for management, and it may potentially be curative.

(250) Submission ID#812646

Development of Cutaneous T Cell Lymphoma in Common Variable Immunodeficiency: Diagnosing the Rash

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Abstract/Case Report Text

Introduction: Common variable immunodeficiency (CVID) is a disorder characterized by impaired immunoglobulin production and frequent or recurrent infections, but also associated with an increased risk for developing malignancies such as lymphomas. Although intravenous and subcutaneous immunoglobin G replacement has been successful in reducing the number of bacterial infections and prolonging survival, it fails to address other complications that arise from this disorder. We report a case of a patient with CVID who developed mycosis fungoides (MF). MF is a rare form of cutaneous T-cell lymphoma, occurring in about 1 in 100,000 to 350,000 individuals, lack of treatment could potentially be fatal.

Case Presentation: A 44-year-old Caucasian woman with a history of ulcerative colitis, allergic rhino-conjunctivitis and tonsillectomy was referred to the immunology clinic for evaluation of low serum immunoglobulins.

There was no family history of infections or immune deficiencies, but paternal grandfather had colon cancer and maternal grandmother had lung cancer. The patient reported frequent episodes of bronchitis, and sinus infections. Immunizations were up to date for her age. Medications included azathioprine, cetirizine, fluticasone nasal spray, hyoscyamine, montelukast, lactobacillus, omeprazole, and olopatadine ophthalmic solution. No history of frequent use of systemic steroids.

Initial serum immunoglobulins revealed normal IgE and IgM but low IgA (72 mg/dL, normal range 81-463 mg/dL) and low IgG (522 mg/dL, normal range 694-1618 mg/dL). CBC with differential, lymphocyte subsets, C3 and C4 levels were normal. While she had adequate protective titers against Haemophilus influenza type b, diphtheria and tetanus, titers against pneumococcus were < 50% protective and she failed to mount an adequate response to pneumococcal polysaccharide vaccine. Given her diagnosis of CVID, she started SCIG (500 mg/kg every 2 weeks). A year later, she developed a bilateral nonpruritic rash in the abdomen and upper trunk. Initial skin biopsy suggested a drug reaction. A subsequent biopsy revealed a superficial perivascular lymphoid infiltrate with focal epidermotropism and positive T cell receptor gamma gene rearrangement, consistent with MF. Testing for cell T receptor Beta gene rearrangement was negative.

While MF treatment consisted of triamcinolone 0.1% ointment, treatment for ulcerative colitis transitioned from azathioprine to vedolizumab.

Conclusions: Cutaneous T cell lymphomas, although uncommon, can be seen in CVID. There are several reasons for the increased risk of lymphoma in CVID. The role of chronic infections and the development of lymphoma as of yet, is not clear. Skin reactions to SCIG products in the areas of infusion are relatively common and resolve promptly. High index of suspicion is crucial in obtaining tissue sample to confirm or rule out malignancy therefore avoiding delaying proper treatment.

(251) Submission ID#812650

LRBA Facilitates Autophagy Through Binding To PIK3R4

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Abstract/Case Report Text

Patients with lipopolysaccharide responsive beige-like anchor protein (LRBA) deficiency present with a plethora of immune related defects including a defective humoral response characterized by low numbers of switched memory B cells and plasma cells, as well as an impaired production of antibodies, leading to recurrent infections. However, the molecular mechanisms behind the defective B cell response remain unknown. To gain better insights into the possible roles of LRBA in B cell physiology, we screened for LRBA-interacting proteins using computational predictions. Twenty-seven proteins involved in vesicle trafficking and autophagy were identified as potential LRBA-interacting partners. To validate those potential LRBA interactions, we performed co-immunoprecipitations and proximity ligation assays (PLA), finding that endogenous LRBA interacts with the phosphoinositide 3-kinase regulatory subunit 4 (PIK3R4) in B cells. PIK3R4 (aka VPS15) is the regulatory subunit of VPS34, the catalytic subunit of the PI3K-III complex, which acts as a positive regulator of autophagy by producing phosphatidyl inositol-3 phosphate (PI(3)P). Autophagy is a catabolic mechanism essential for cell survival and plasma cell differentiation. In fact, we observed that reduced LRBA impaired the production of PI(3)P upon autophagy induction. In addition, we observed in both LRBA-deficient HeLa and B cells reduced mobility, abnormal accumulation and increased size of autophaghosomes, accompanied by an atypical lysosomal positioning. These abnormalities are due to a blockade of the autophagosome-lysosome fusion, as detected by reduced LC3-II lipidation upon autophagy induction in the presence of lysosome inhibitors. Interestingly, LRBA-deficient HeLa and B cells exhibited enhanced activity of mammalian target of rapamycin complex 1 (mTORC1) signaling, a key suppressor of autophagy whose activation possibly contributes to defective autophagy. Taken together, B lymphocytes lacking LRBA can form autophagosomes but they fail to fuse with lysosomes. Thus, we

propose a role of LRBA at late stages of autophagy through the binding to PIK3R4.

(252) Submission ID#812659

Eosinophilic esophagitis in patients with Phosphoinositide 3-Kinase p110δ

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Abstract/Case Report Text

APDS caused by gain-of-function mutations (GOF) in the genes (PIK3CD and PIK3R1), encoding for the p110δ and p85 subunits of phosphoinositide 3-kinase δ (PI3Kδ), results in hyperactivation of the PI3K/AKT/mTOR/S6K pathway and lead to immune dysregulation, lymphoproliferation and immunodeficiency. APDS manifests with respiratory tract infections, bronchiectasis, susceptibility to Herpes group viruses, autoimmunity, cytopenia, lymphoproliferation and lymphoma. Gastrointestinal system manifestations include enteropathy, colitis, and liver disease. Eosinophilic esophagitis (EoE) or eosinophilic gastrointestinal disease (EGD) have been under diagnosed in reported APDS cohorts.

Objectives: To review the incidence, demographics and relevant clinical data for eosinophilic gastrointestinal disease in a single center APDS cohort.

Methods: Review of clinical and laboratory findings from 70 APDS patients followed at the NIH Clinical Center, from 2005 to 2019.

Results: 12 patients were either historically diagnosed or actively studied at our center for EGD. Incidence of all EGD is 17 % in our cohort and all patients had mutations in PIK3CD, none in PIk3R1. Most patients also had multiple GI manifestations.

Conclusion: Immunopathology and genetic predisposition leading to EoE is complex. Eosinophilic GI disease including EoE appears to represent significant GI pathology in APDS. This implies that activation of PI3K pathway may be directly involved in the etiology of EoE.

(253) Submission ID#812666

A Novel Gain of Function Mutation in NLRC4 with Phenotypic Variability in 3 Siblings

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Abstract/Case Report Text

Introduction: Activation of the NOD-like receptor family CARD-containing 4 protein (NLRC4) leads to the formation of an inflammasome. The inflammasome, a large cytosolic multiprotein complex of innate immunity, promotes proteolytic cleavage, maturation, and secretion of pro-inflammatory cytokines, including IL-1 and IL-18. A gain-of-function mutation (GOF) in the gene encoding NLRC4 or NLRC4-inflammasomopathy is characterized by hyperinflammation with persistent elevated IL-18, infantile enterocolitis, and early-onset macrophage activation syndrome (MAS).

Objectives: To describe phenotypic variation among three siblings with a novel NLRC4 GOF variant and to expand our current understanding of the clinical manifestations of the disease

Methods: Clinical and laboratory features were studied in three siblings of a Hispanic family with a novel NLRC4 GOF variant.

Results: A novel variant, c.1475G>A (p.Arg492Gln) on the NLRC4 gene, was identified in a 3-year-old male with recurrent febrile episodes since one year of age. His laboratory findings showed highly elevated ESR, CRP, IL-18, and fecal calprotectin. His endoscopic finding was unremarkable. The recurrent fever partially responded to canakinumab. A 10-year-old sister with ileocolonic Crohn's disease for two years was found with the same NLRC4 variant and highly elevated IL-18. Crohn's disease was well controlled after adding infliximab infusion to methotrexate therapy. A15-year-old sister, who has been asymptomatic and healthy, was tested with the same positive NLRC4 variant and highly elevated IL-18. The NLRC4 variant is inherited from their father, who currently has a diagnosis of psoriasis vulgaris. The IL-18 levels of the three siblings show in the Figure 1.

Conclusions: We report a novel c.1475G>A (p.Arg492Gln) variant in the NLRC4 gene causing persistent elevated IL-18 and autoinflammatory syndrome with recurrent fever and Crohn's disease. The clinical manifestations of the same variant may be variable even in the same family. In addition to the early onset of enterocolitis and MAS, the NLRC4-inflammasomopathy should be

considered in children with unexplained recurrent fever episodes and persistent high IL-18.

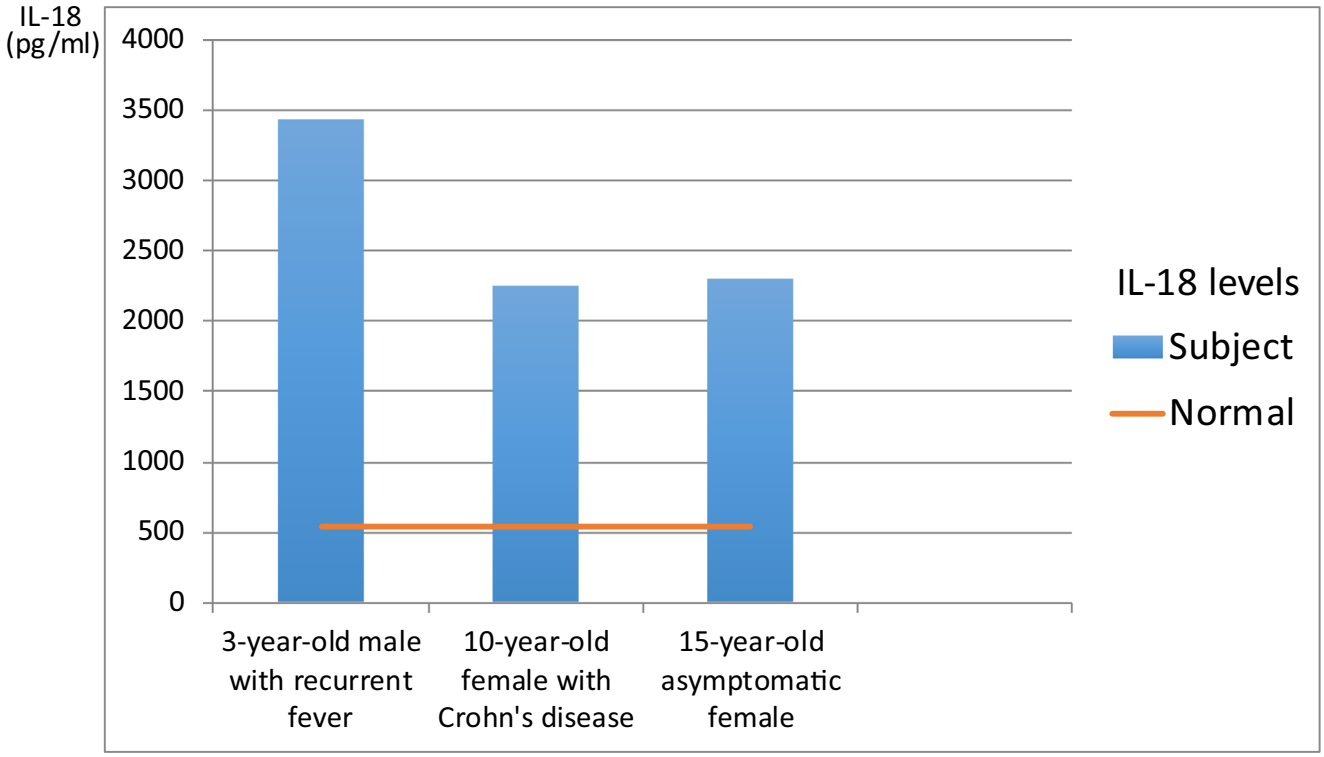

Fig 1. IL-18 levels of the three siblings with a novel variant, c.1475G>A (p.Arg492Gln) on the *NLRC4* gene

(254) Submission ID#812671

Outcome Of Hematopoietic Stem Cell Transplantation In A Case Of Familial Hemophagocytic Lymphohistiocytosis With A Syntaxin 11 Gene Mutation, A Non-T-Cell-Depleted Graft From An Haploidentical Donor

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Abstract/Case Report Text
Familial Hemophagocytic Lymphohistiocytosis type 4 (FLH-4) is an autosomal recessive disease, that arises from the mutation of STX11. Patients with FLH-4 develop the classic HLH phenotype early in life, with periods of remission. The pathogenesis is associated with a defect of perforin-dependent cytotoxicity. T-CTL and NK cells fail to remove abnormal cells, consequently, an uncontrolled proliferation and activation of CD8+ T cells and macrophages develops and generates an inflammatory cytokine storm and a solid organs infiltration. Mortality of FHL-4 is very high without treatment; allogeneic HSCT is the only curative therapy. We report the outcome of a child with FHL-4, four years after his treatment with an allogeneic HSCT using an HLA haploidentical donor. Case report: A boy born in December 2007, previously healthy, the only child of parents without consanguinity. He has a normal family and perinatal history. When he is 7 years old, he begins suffering from with fever, arthralgia, hepatosplenomegaly and pancytopenia. He meets the diagnostic criteria for Hemophagocytic Lymphohistiocytosis (HLH) and received treatment with iv methylprednisolone and entered in remission of its HLH. Six months later, he restarts his clinical picture of HLH and

receives treatment with the HLH-2004 protocol, which leads to remission. A year later he presents a new episode of HLH that responds to IVIg, cyclosporin A and dexamethasone. The possibility of primary HLH is then suggested. When he is 8 years old, the molecular diagnosis is made with the identification of a mutation in the STX11 gene, the case was classified as FHL-4 due to Syntaxin 11 deficiency. In October 2015, a HSCT was performed. At the age of 12, his clinical status is evaluated and laboratory studies show that his immunodeficiency due to Syntaxin 11 deficiency was cured. His 42-year-old mother, HLA haploidentical was used as a donor and a protocol developed for the treatment of Osteopetrosis was applied. The HSCT protocol did not use a T-cell depleted graft. HSTC conditioning was done with Melphalan days -11 and -3, Fludarabine days -7 to -2, Anti-thymocyte globulin days -7 and -6, and Cyclophosphamide day -2. Prophylaxis against graft vs host disease was with Tacrolimus, Methylprednisolone, Methotrexate and Mycophenolate mofetil. Engraftment was detected at day +14, no evidence of graft vs host disease was detected and he leaves the hospital at day +35. Outcome: Four years after the HSCT, he is a healthy 12 years old child, which has not experienced new episodes of HLH. A quimerism test showed 100% of hematopoiesis from the donor. The hemogram, a lymphocyte distribution in peripheral blood and a phytohemagglutinin blastic stimulation were normal. There is no clinical evidence of graft vs host disease. His length and weight are normal for his age, and he attends school at the level for his age.
Conclusions: The use of HSTC protocols with haploidentical donor and a non-T-cell-depleted graft is an effective therapy for FHL-4 cases. The outcome of the treatment shows a complete immunological reconstitution and the absence of graft vs host disease.

(255) Submission ID#812673

Characterization of Cytopenias in Primary Immunodeficiencies in the USIDNET Registry

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Abstract/Case Report Text
Background: Patients with primary immunodeficiencies (PID)s often present with cytopenias, either as a feature of the underlying disease or as a complication. We sought to identify and characterize the PID patients with and without reported cytopenias enrolled in the United States Immunodeficiency Network (USIDNET) registry.
Methods: We included all the subjects enrolled in the entire USIDNET registry (n=4005) through April 2019. PID diagnoses were categorized using 2017 International Union of Immunological Societies (IUIS) categories. Patients were categorized with cytopenia if they had one or more of the following diagnoses: such as pancytopenia, lymphopenia, neutropenia, monocytopenia, anemia, and thrombocytopenia. Those with and without any reported cytopenias were compared using chi2 tests (Fisher's exact) for categorical data and continuous data were compared using non-parametric tests (Mann Whitney Wilcoxon rank sum test).
Results: The USIDNET registry had 4005 individuals with a median age of 19 years (interquartile range 8-41 years), and the median age at diagnosis was 8 years (interquartile range 1.4 to 29.9 years). About one-third (30.6%, 1224/4005) individuals had at least one reported cytopenia and 7.8% (314/4005) had at least one reported immune cytopenia. The registry had 53.6% (2003/3735) males and 46.4% (1732/4005) females with a similar distribution among those with reported cytopenias. Most patients identified as White (86%, 2674/3099) and Black (7%, 206/3099) with a similar distribution among those with and without cytopenia. The majority of individuals (88%, 1986/2253) were alive at last visit; however, those with reported cytopenia (19.7%, 161/816) had a higher mortality than those without cytopenia (7.4%, 106/1437); ($p < 0.001$). Subjects with reported cytopenia as compared to those without were more likely to have received immunoglobulin replacement (77% vs 70.5%, $p < 0.001$). Individuals in IUIS diagnostic categories I (14.1%, 172/1215), II (15.2%, 185/1215), III (44.8%, 544/1215) and IV (11.2%, 136/1215) accounted for the majority (85%) of diagnoses in those subjects with cytopenia. The individual diagnoses most often reporting cytopenias included CVID (34.9%), SCID (8.3%), CGD (6.4%), Wiskott Aldrich syndrome (5.8%) and DiGeorge syndrome (5.4%). Most commonly reported types of cytopenias included anemia (17.2%, 689/4005), thrombocytopenia (11.9%, 476/4005), neutropenia (7.7%, 308/4005) and lymphopenia (6.3%, 254/4005). Overall reported frequency of malignancies in the registry was 7.6% (304/4005). However, more malignancies were noted among those with reported cytopenias (11.8%, 145/1224) as compared to those without reported cytopenias (5.7%, 159/2781); ($p= < 0.001$), which was a similar effect when both hematologic and non-hematologic malignancies were assessed separately. Among those with and without reported immune cytopenias, there was a higher frequency of hematologic malignancy (6.7% vs 3%, $p < 0.001$).
Conclusions: Cytopenias are commonly reported among all subjects with PIDs in the USIDNET registry; however, differences are seen in the frequencies of reported immune and non-immune cytopenias within specific diagnoses. Individuals with PIDs and cytopenias (both immune and non-immune) may have higher mortality and frequency of malignancy as compared to those individuals without reported cytopenias. A high index of suspicion and close monitoring may be warranted in this potentially higher risk group of PID individuals with cytopenias.

(256) Submission ID#812799

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Abstract/Case Report Text
Background: Chronic granulomatous disease (CGD) is one of the more common primary immunodeficiencies (PIDs) in the Asian Primary Immunodeficiency Network (APIN).
Objectives: To describe and analyze the epidemiology, genotype, phenotype and clinical care of chronic granulomatous disease in the Asia Pacific and beyond.
Methodology: Targeted Sanger sequencing was carried out on 190 referred potential CGD families, followed by whole exome sequencing when needed in 7 families. Clinical notes of 90 patients

from referrers have been retrieved and could be deep-phenotyped manually using human phenotype ontology (HPO) vocabulary. Analysis of 12 non-overlapping Asian CGD cohort studies identified on PubMed was conducted to study the infection profiles. An online survey was sent to all APIN referring doctors to investigate clinical practices for CGD.
Results: Preliminary data from the ongoing project are available. Sanger sequencing resulted in diagnoses of 104 CGD patients, 78 of them X-CGD and 26 AR-CGD, and 50 X-CGD carriers. Follow-up whole exome sequencing for other suspected CGD or PID patients failed to diagnose any additional cases of CGD despite a review for CYBC mutations after its discovery. Genotype-wise, there are 78 CYBB, 9 CYBA, 13 NCF1 and 4 NCF2. Preliminary HPO analysis showed the top 3 phenotypic abnormality HPO terms are abnormality of the respiratory system HP:0002086 (78%), abnormality of the digestive system HP:0025031 (73%) and abnormality of the integument HP:0001574 (49%). AR-CGD is associated with fewer applicable phenotypic abnormality HPO terms and fewer applicable total HPO terms under the phenotypic abnormality subontology by linear regression. Independent samples T test with selected individual HPO terms revealed that AR-CGD is significantly and negatively associated with infection following live vaccination HP:0020085, sepsis HP:0100806 and perianal abscess HP:0009789. In the 12 Asian cohort studies on CGD, the top 3 implicated microbial genera are mycobacteria (38%), staphylococci (14%) and Aspergillus (14%). Ecologic analysis revealed that mycobacterium tuberculosis infection and Candidiasis correlate with higher annual precipitation and higher average temperature of the study site. 18 responses to the CGD care survey have been collected from 13 centers in the Asia Pacific and 3 centers in Africa. The centers have collectively diagnosed 451 CGD patients, including 12 X-CGD carriers with carrier disease and 11 adult CGD patients. Among the 16 centers, biochemical CGD diagnostics (nitroblue tetrazolium test or dihydrorhodamine 123 test) are available at 10 only. While most centers offer septrin and itraconazole as prophylactic medication for CGD, 1 center offers itraconazole only and 1 septrin only. One elected to prescribe IFN-gamma in addition. Only 6 centers have performed hematopoietic stem cell transplants for CGD.
Reference
1. P. P. Lee, Y. L. Lau, Improving care, education, and research: the Asian primary immunodeficiency network. Ann N Y Acad Sci 1238, 33-41 (2011).

(258) Submission ID#812919

Title: Patient and Clinical Characteristics of a Large US Sample of Patients With Primary Immunodeficiency Diseases (PID) Initiating Subcutaneous Immunoglobulin (SCIG) Therapy

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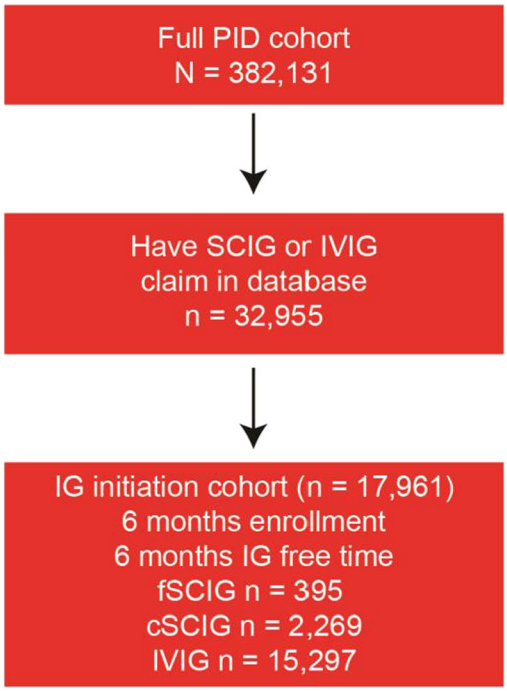
*Affiliation at the time of the study

INTRODUCTION/BACKGROUND
Primary immunodeficiency diseases (PID) are a group of rare, heterogenous disorders caused by an array of genetic abnormalities that impair the immune system. Immunoglobulin (IG) replacement therapy (IGRT) is standard first-line treatment for most forms of PID with defective antibody production. Subcutaneous IG (SCIG) is an increasingly popular route of administration with different options, from highly concentrated 20% IG (cSCIG) to facilitated 10% IG (fSCIG) delivered with Recombinant Human Hyaluronidase. Patient and clinical characteristics between PID patients and those who are incident SCIG users are poorly understood.
OBJECTIVE: Identify and describe demographic, clinical, and treatment characteristics of patients with PID in the US and those initiating SCIG treatment.
DESIGN/METHODS: This claims-based cohort study identified PID patients and PID patients who were new users of IGRT from 2012–2018 via diagnosis codes in IBM® Watson HealthTM MarketScan® Research Databases (Figure). Clinical and demographic characteristics, including the use of a novel claims-based weighted algorithm (Risk Vital Sign; RVS) and those initiating IVIG and SCIG, were described. Stratified analysis based on PID diagnosis codes was performed. Probability of receiving available IG treatments based on baseline characteristics was evaluated by logistic regression and propensity score methods.
RESULTS: Selected clinical and demographic characteristics, severity measures, and previous treatments between the overall PID population (n=382,131), PID patients initiating SCIG (n=2,604), and PID patients initiating IVIG (n=15,327) are presented in the table. Patient characteristics and previous treatments tended to be stable, although hypertension, obesity, and corticosteroid use increased during the study period. New IG users tended to be older and female, with increased depression, dyslipidemia, and hypertension than all PID patients. New SCIG users had more diagnoses of respiratory (e.g., asthma, COPD) and inflammatory (e.g., arthritis, fibromyalgia, inflammatory bowel disease) comorbidities and less cancer than all PID patients. New SCIG users compared with new IVIG users had increased asthma and COPD, fibromyalgia, and inflammatory bowel disease and decreased cancer and peripheral vascular disease.
Previous corticosteroid use was higher in IG users than all PID patients. Among SCIG users, prior PID treatments of IV antibiotics, and oral high potency antibiotics were similar to all PID patients. IVIG users had higher IV antibiotic and antifungal use.
RVS was initially developed to identify patients likely to have undiagnosed PID. This analysis applied RVS to patients diagnosed with PID to assess severity. RVS based on 1-year history in the overall PID cohort was predominantly Low, with only 18.0% of patients scoring in the Medium and High ranges. RVS was increased in incident IG users, with (37.4% Medium/High for SCIG; 46.4% Medium/High for IVIG). In other markers of severity, SCIG users had more sinusitis and IVIG had more pneumonia than all PID. IG users had fewer abscesses, cellulitis, and otitis media than the full PID cohort.
CONCLUSIONS: This exploratory analysis showed a trend toward increased hypertension, inflammatory and respiratory comorbidity, higher RVS, and previous corticosteroid treatment in patients initiating on IG compared with all PID patients. Results could be confounded based on PID diagnosis codes used and warrants further research.
Author disclosures: **MP**, **CAS**, and **ZH** are employees and stockholders of the Takeda group of companies. **JO** is a consultant to Takeda. **JBL** is an employee of RTI Health Solutions, an organization funded by Takeda to conduct this research. **MER** was an employee of RTI Health Solutions at the time this research was conducted.

Presenting author: Colin Anderson-Smits
Submission topic: Immunoglobulin Replacement Therapy



cSCIG, 20% subcutaneous immunoglobulin; fSCIG, facilitated subcutaneous immunoglobulin; IG, immunoglobulin; IVIG, intravenous immunoglobulin; PID, primary immunodeficiency diseases; SCIG, subcutaneous immunoglobulin

Table 1 Clinical and demographic characteristics, severity measures, and previous treatments for the PID cohort, SCIG initiators, and IVIG initiators

Characteristics	PID Cohort (N = 382,131)	SCIG Initiators (n = 2,604)	IVIG Initiators (n = 15,327)
Age, median (IQR)	46 (34)	48 (34)	54 (30)
Sex, n (%)			
Female	226,785 (59.3)	1,787 (68.6)	8,753 (57.1)
Male	155,346 (40.7)	817 (31.4)	6,574 (42.9)
Geographic region, n (%)			
North Central	66,736 (17.5)	406 (15.6)	3,022 (19.7)
Northeast	69,027 (18.1)	302 (11.6)	2,179 (14.2)
South	128,933 (33.7)	1,056 (40.6)	5,018 (32.7)
West	51,425 (13.5)	353 (13.6)	2,168 (14.1)
Unknown	66,010 (17.3)	487 (18.7)	2,940 (19.2)
Insurance type, n (%)			
Commercial	278,385 (72.9)	1,877 (72.1)	9,658 (63.0)
Medicaid	60,010 (15.7)	456 (17.5)	2,765 (18.0)
Medicare supplementary	43,736 (11.4)	271 (10.4)	2,904 (19.0)
Clinical characteristics, n (%)			
Arthritis	87,755 (23.0)	797 (30.6)	4,757 (31.0)
Asthma	96,062 (25.1)	1,533 (58.9)	5,804 (37.9)
Cancer	50,405 (13.2)	282 (10.8)	3,350 (21.9)
Cerebrovascular disease	42,202 (11.0)	348 (13.4)	2,694 (17.6)
COPD	143,829 (37.6)	1,852 (71.1)	8,928 (58.3)
Cytomegalovirus	5,556 (1.5)	32 (1.2)	769 (5.0)
Depression	72,114 (18.9)	792 (30.4)	4,255 (27.8)
Diabetes	73,839 (19.3)	526 (20.2)	3,970 (25.9)
Dyslipidemia	144,892 (37.9)	1,160 (44.6)	7,509 (49.0)

(continued)

Dysthymia	17,986 (4.7)	224 (8.6)	1,093 (7.1)
Fibromyalgia	51,253 (13.4)	642 (24.7)	2,992 (19.5)
Food allergy	6,478 (1.7)	91 (3.5)	264 (1.7)
Gout	21,573 (5.6)	141 (5.4)	1,071 (7.0)
Hypertension or coronary artery disease	159,651 (41.8)	1,176 (45.2)	8,890 (58.0)
Hepatitis B	2,730 (0.7)	11 (0.4)	113 (0.7)
Hepatitis C	7,767 (2.0)	15 (0.6)	176 (1.2)
HIV	5,779 (1.5)	12 (0.5)	112 (0.7)
Hypothyroidism	76,422 (20.0)	738 (28.3)	4,112 (26.8)
Immune-mediated arthritis	39,191 (10.3)	255 (9.8)	1,102 (7.2)
Inflammatory bowel disease	21,608 (5.7)	318 (12.2)	1,214 (7.9)
Juvenile idiopathic arthritis	2,784 (0.7)	28 (1.1)	83 (0.5)
Leukemia	18,790 (4.9)	52 (2.0)	3,557 (23.2)
Lupus	15,159 (4.0)	127 (4.9)	625 (4.1)
Lymphoma	26,369 (6.9)	123 (4.7)	4,350 (28.4)
Myocardial infarction	13,910 (3.6)	94 (3.6)	853 (5.6)
Obesity	59,906 (15.7)	569 (21.9)	2,896 (18.9)
Peripheral vascular disease	38,981 (10.2)	274 (10.5)	2,500 (16.3)
Pulmonary embolism	9,080 (2.4)	91 (3.5)	745 (4.9)
Deep vein thrombosis	18,858 (4.9)	162 (6.2)	1,518 (9.9)
Psoriasis	12,883 (3.4)	97 (3.7)	455 (3.0)
Scleroderma	2,457 (0.6)	15 (0.6)	148 (1.0)
Markers of PID severity, n (%)			
Risk vital sign			
Low	313,611 (82.1)	1,630 (62.6)	8,217 (53.6)
Medium	15,104 (4.0)	196 (7.5)	916 (6.0)
High	53,416 (14.0)	778 (29.9)	6,194 (40.4)
Abscess	47,709 (12.5)	125 (4.8)	1,197 (7.8)
Bacterial pneumonia	75,321 (19.7)	590 (22.7)	4,362 (28.5)
Cellulitis	57,282 (15.0)	187 (7.2)	1,438 (9.4)
Lymphadenitis	33,690 (8.8)	107 (4.1)	1,467 (9.6)
Mastoiditis	674 (0.2)	7 (0.3)	27 (0.2)
Osteomyelitis	5,531 (1.4)	23 (0.9)	176 (1.2)
Recurrent otitis media	74,887 (19.6)	337 (12.9)	1,374 (9.0)
Recurrent sinusitis	157,932 (41.3)	1,531 (58.8)	5,861 (38.2)
Prior PID treatments, n (%)			
IV antibiotics	95,129 (24.9)	601 (23.1)	4,497 (29.3)
High-potency oral antibiotics	130,153 (34.1)	967 (37.1)	4,720 (30.8)
Systemic high-dose corticosteroids	186,557 (48.8)	1,380 (53.0)	10,196 (66.5)
Antifungals	81,737 (21.4)	656 (25.2)	4,520 (29.5)
Stem cell transplant	3,137 (0.8)	1 (<0.1)	385 (2.5)
Interferon-gamma therapy	6 (<0.1)	0	0
Growth factors	16,421 (4.3)	16 (0.6)	1,569 (10.2)

COPD, chronic obstructive pulmonary disease; HIV, human immunodeficiency virus; IQR, interquartile range; IV, intravenous; IVIG, intravenous immunoglobulin; PID, primary immunodeficiency diseases; SCIG, subcutaneous immunoglobulin.

Figure 1. Attrition Due to Application of Eligibility Criteria for the IG Initiation Cohort

(259) Submission ID#812938

Vascular abnormalities found in Coronary and Cerebral Arteries in LOF STAT3

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Abstract/Case Report Text
Introduction: Dominant negative mutations in STAT3 (LOF STAT3; Job's syndrome) cause a primary immune deficiency characterized by eczema, recurrent skin and lung infections, and connective tissues and skeletal abnormalities. Over the last several years, vascular abnormalities causing tortuous and aneurysmal middle-sized arteries have increasingly been recognized. Our institution has been imaging prospectively the coronary and cerebral arteries since 1999 – 2019 for brain imaging and from 2004-2019 for heart imaging. The purpose of this review is to provide an update on the extent of clinical manifestations noted in HIES

patients in a larger cohort group and to determine if there are patterns of disease not previously reported.
Methods: We performed a retrospective chart review of patients with LOF STAT3 (n=81) followed at the National Institutes of Health. We specifically looked at tortuosity, aneurysms, and dilation of both coronary and cerebral arteries. Epidemiologic information, STAT3 mutation, co-morbidities, and laboratory information were reviewed along with imaging studies, specifically Brain MRI, Brain MRA, Heart MRI, and Coronary CT.
Results: Most recently, patients with HIES are found to have vascular abnormalities including tortuosity, dilatation, narrowing, and aneurysms of middle sized, cerebral, and coronary arteries. In an effort to determine the extent of vascular involvement in addition to miscellaneous organ involvement, we are reviewing a cohort of 81 patients with HIES who were evaluated at the NIH. Of these 81 patients, 53 are women and 28 are men. Of these 81 patients, two have passed away due to vascular events leading to their deaths. There are four patients under the age of 20, 43 patients between the ages of 21 and 40, 33 patients between the ages of 41 and 60, and three patients above the age of 60 (age range 15-68, mean age 35.6).
Of the 81 patients, five of these patients were found to have abnormal Brain MRI/MRA at an approximate rate of 6.2%. Two of these patients were found to have at least one cranial aneurysm, two of these patients were found to have a level of narrowing or stenosis, and one patient was found to have dilatation.
In terms of coronary abnormalities, 36 of the 81 (44.4%) patients were noted to have at least one coronary abnormality including dilatation, aneurysm, or tortuosity on Heart MRI or Coronary CT. Eight patients (9.8%) were found to have dilatation of which four patients were female and 4 patients were male. Of the 81 patients, ten patients (12.3%) were found to have at least one aneurysm. There were 30 patients (37%) that were found to have at least mild tortuosity.
Conclusion: Vascular abnormalities in our LOF STAT3 patients occurred at an exceedingly high rate- cerebral and coronary artery, 6.2% and 44.4% respectively. Due to this, patient's with LOF STAT3 should be considered for screening with brain and heart imaging. Currently, there are no guidelines which outline the appropriate timeline for screening in these patients however following these patients over time will allow us to determine the most appropriate interval for imaging follow up.

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PIK3CD VUS In A Patient With Common Variable Immunodeficiency

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Abstract/Case Report Text
63 year old woman with personal history of severe and recurrent upper and lower respiratory infections, chronic pulmonary disease with bilateral bronchiectasis and several micro nodules, chronic diarrhea without diagnosis (colonoscopy with mild colitis, without CMV. No bacterias no parasits were found in stools), mild osteopenia, focal lesion in right hepatic lobe, atopic dermatitis and anemia. She was followed up in other center and in 1996 she was diagnosed with common variable immunodeficiency (CVID) and started treatment with intravenous immunoglobulin (IVIG), but with low adherence to it. She did not have referred history of lymphoproliferation nor significant viral infections. She had a daughter with spherocytosis who required esplenectomy and also had bronchiectasis and CVID diagnosis, she deceased at 28 years old due to pulmonary infection. One 30 years old son has anemia. Her other daughter and son are healthy.
In our first immunologic assessment we found severe hypogammaglobulinemia with absence of B cells in peripheral blood. She started with high doses of IVIG (800 mg/k/month) and antibiotic prophilaxis with improvement of the functional respiratory test and without new infections.
Thinking that her clinical picture could be other than CVID we order a genetic study. A Nextera Exome Capture and Next Generation Sequence with Illumina HiSEq was made and an heterozygous VUS in PIK3CD gene (chr1:9. 775. 746, p. P97A) was found. Her son with anemia also have the same variant. (His immunological studies are still pending).
Now a days she is stable, without infections with IVIG and antibiotic profilaxis but because of the chronic diarrhea and the pulmonary compromise, she began with sirolimus but it was suspended because severe intolerance (vomiting and diarrhea that caused dehydration). In plan to iniciate treatment with mycophenolate mofetil.
Conclusion: Clinical presentations of primary immunodeficiencies are becoming more complex, and their diagnosis imply a challenge for immunologist nowadays. Studies with Next Generation Sequence is a very useful tool in undefined cases, especially when more than one member in the family are involved.

(261) Submission ID#813032

Bortezomib for Treatment of Disseminated Nontuberculous Mycobacteria in a Patient with Anti-IFN-γ autoantibodies

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Abstract/Case Report Text
Autoantibodies to interferon- γ (IFN-γ)are associated with disseminated nontuberculous mycobacterial (NTM). We have previously published our experience with treatment of refractory infection with rituximab. We report a case of recurrent infection after initial response and treatment with rituximab targeting CD20+ cells and progression of infection despite repeated treatment with rituximab and subsequent successful treatment with bortezomib.
Patient is 38 years old, Philippine female with history of IFN-γ autoantibodies and refractory disseminated Mycobacterium avium complex (MAC). At 32 yrs. she presented with lower back and cervical pain, despite conservative therapy, did not improve, and was associated with lymphadenopathy and severe myalgias. With the addition of steroids, the

pain improved but rapidly worsened with tapering of steroids. The PET/CT at that time, showed extensive hypermetabolic areas in lymph nodes, femurs, left acetabulum, left pubic ramus, right ischium, sacrum, both iliac bones, eight rib, T4 and T8, both clavicles, both humeral, manubrium, and extension into musculature. Initial biopsies were culture negative, 16S was positive for MAC. She was referred to the National Institutes of health where she was diagnosed with autoantibodies to interferon- γ .

Prior to referral, she initially was treated with azithromycin, ethambutol (need to discontinue due adverse effects), amikacin, rifampicin, linezolid, with not no evidence of clinical response. She underwent debridement of epidural anterior abscess to T8. Surgery involved T8-9 laminectomy, and curettage on several bones. She presented unable to walk secondary to pain and neuropathies.

Initial Laboratory:

CRP:90mg/L; WC 13.51; Hgb: 8.2g/dL; ANA :4.6(strongly positive) CD20: 373 uL

Her first course of rituximab consisted of 7 doses of 1gm at D0, 14, 42 and monthly thereafter for a total of 7 doses with clinical and radiographic improvement. She was maintained on optimal antibiotics therapy – meropenem, rifampin, azithromycin, moxifloxacin, and clofazimine. Two years after she completed rituximab, she presented to her home hospital with increased left hip pain and biopsy grew MAC. Retreatment with rituximab failed to show clinical improvement. Her medical regimen was augmented with tedizolid and bedaquiline, however no IV antibiotics were added. Rituximab was reinitiated, after the progression of symptoms despite treatment with rituximab; Bortezomib, was trialed using the schedule based on the multiple myeloma literature. She completed 5 full cycles (two at the NIH, three at home). She subsequently has had clinical improvement and is working again and has not had progressive neurologic decline. The titers of antibodies to interferon-· did not follow the clinical improvement. However, we plan to keep her CD20+ cells zero and continue the bortezomib given her clinical and radiologic improvement.

(262) Submission ID#813107

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Abstract/Case Report Text

For patients with primary immunodeficiencies (PID), finding a genetically defined diagnosis can be critical for prognosis, treatment, and counseling. However, for many patients, determining a genetic etiology remains elusive despite routine gene panel and exome sequencing because of an inability to resolve variants of uncertain significance (VUS). CRISPR-based genome editing could be used to address this need by introducing patient-derived VUS into primary human immune cells for further study; however, existing techniques are limited by poor efficiency. We recently developed novel non-viral techniques for large gene editing in primary human T cells and hematopoietic stem cells (HSCs). We achieve up to 8-fold greater efficiency than existing tools using CRISPR Cas9 ribonucleoprotein nanoparticles that are non-covalently linked to homology directed repair (HDR) template DNA. Whereas mutation analysis has previously been limited to expensive and time-consuming animal models and transformed cell lines, we now have the ability to rapidly recreate any mutation in the native gene locus in otherwise healthy primary human cells.

We demonstrate this ability by using our technique to knock-in well characterized loss-of-function mutations in JAK3 and IL2RG and gain-of-function mutations in JAK3 that are known to cause severe combined immunodeficiency and tumor growth, respectively. We show that these recreated mutations have the expected effects on T cell proliferation and intracellular signal transduction in the setting of IL-2 stimulation.

We then use our technique to investigate a prototype case of an adult patient with the unusual combination of common variable immunodeficiency, inflammatory arthritis and uveitis, and neutrophilic urticaria. Genetic testing in this patient had previously revealed heterozygous coding VUS's in four genes previously associated with a PID disease, including JAK3, but none of the specific variants have been previously reported. It is thus not clear which mutation (if not more than one) causes this patient's dysregulated cell activation, which limits targeted treatment options with kinase inhibitors or future gene or cell therapy.

By knocking our patient's JAK3 variant into primary human T cells and comparing these cells to those carrying wild-type, known loss-of-function, and known gain-of-function mutations, we are able to rapidly characterize the functional impact of our patient's variant and isolate its effect on his complex phenotype. This in vitro genetic engineering approach thus allows patient-specific VUS to be modeled directly in primary human immune cells with a rapid turnaround time that is relevant for clinical applications, including molecular diagnosis and screening of pharmacologic or gene therapies. Further, similar strategies could be leveraged as a potential basis for future gene correction therapy.

(263) Submission ID#813425

Variant of Ataxia Telangiectasia in Colombia Caused By New Splicing Mutation Not Reported In The Literature

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Abstract/Case Report Text

Definition : "AT variants" comprise a heterogeneous group characterized by the later onset of clinical symptoms, a slower progression, a prolonged lifespan compared to most patients with AT and decreased levels of chromosomal instability and cellular radiosensitivity. In these patients, telangiectasia and / or immunodeficiency may be absent, while neurological features are present.(1).

Material and methods :A 4 years old girl, born out of a non- consanguineous parents with clinical picture consisting in progressive alteration of the march (15 months), associated to exotropia, no weight gain or height, alteration of balance while she is sitting, she walks by herself. She has 1 acute bronchiolitis.

Results and Discussion: Brain magnetic resonance without alterations. Abdominal ultrasonography and CPK normal. Ophthalmologist assessment found exotropia, He did not find telangiectasia.

Motor and sensitive neuro-conduction were reported normal. Alpha fetoprotein 102 ng / ml increased.

karyotype XX, non-structural alterations. Normal auditory-visual evoked potentials. Whole exome sequencing (WES): identified the small homozygous pathogenically deletion ¬c.5496 + 2_5496 + 5del TAAG; p.? have a spelling effect in the splicing. it is not present in the population database or not as a known variant in

the general population. IgG antibodies for hepatitis B 31.83 iu / l. rubella IgG> 500. LTCD4 169 , LTCD8 62 , LTCD3 247. CD4 / CD8: 2.73 , lymphocytes B 161 , natural killer 314 cell/mm3.
Conclusions: Although the ATM activity corresponding to this new splicing mutation is unknown, it is presumed that it has some residual function, since splicing mutations is associated with better neurological prognosis have been reported. (2)

(264) Submission ID#808024

Recurrent Respiratory Viral Infections in a 22q11.2 Deletion Syndrome Patient

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Abstract/Case Report Text
Background: The 22q11.2 deletion syndrome (22qDS) is a common syndrome occurring in about 1: 4000 births. The most common phenotypic features are cardiac anomalies, palatal dysfunction, dysmorphic facial features, and hypoplastic parathyroid glands and thymus. The main immune deficiency manifestation is T cell lymphopenia, ranging from complete absence of T cells to normal T cell counts with various functional affectations. Additionally, humoral dysfunction can be associated with recurrent infection or autoimmunity. Thus far, intravenous immune globulin (IVIg) has been used for 22qDS patients with recurrent bacterial infections in whom low antibody levels or poor vaccine responses were found. However standard management for recurrent viral respiratory infections is currently not available.
Objective: To describe unique management of recurrent viral respiratory infections in a 22qDS patient. Case: 5-year-old male with a history of 22qDS complicated by truncus arteriosus, VSD, hypoparathyroidism, asthma, and autism was followed for a history of "frequent pneumonias." He had daily rhinitis and a history of frequent otitis media which resolved after tympanostomy tube placement at age 3y. However, over the next two years, he had 11 admissions with various respiratory viral infections resulting in respiratory distress and prolonged oxygen need. Viruses detected during these separate admissions varied and included Parainfluenza 3, Metapneumovirus, RSV, B and Coronavirus NL63, Coronavirus HKU1, and Rhino/Enterovirus (5 times total). He was previously on a prophylactic course of antibiotics which made no difference in his symptoms.
Laboratory evaluation showed protective adaptive immunity with normal immunoglobulin numbers for age (IgG 589 mg/dL), along with normal B and T cell numbers. He had protective pneumococcal titers and mounted a normal mitogen response. While his NK cell numbers were normal, his NK T cells were low. His TLR functioning also appeared normal.
In order to decrease his overall illness burden and to keep him out of the hospital, IVIg infusions (~500mg/kg) were initiated monthly. Shortly after initiation of treatment, his nasal purulence and drainage resolved and his family noted that he became more active and playful. Treatment was continued for 18 months, during which time he had only one episode of influenza infection needing inpatient management. He has been off of IVIg for 12 months without recurrence of his viral infections. Conclusion: In this patient, severe recurrent viral respiratory infections despite apparently normal adaptive and cellular immunity presents a unique management dilemma. This was not an issue of recurrent bacterial infections as prophylaxis did not make a difference in the frequency of his infections. His NK T cells were low, which could have contributed to his frequent viral infections as NKT cells are known to play a role in viral immunity. The successful use of IVIg treatment in his case points to a different use for IVIg, namely for the anti-respiratory virus antibodies which are presumably contained within the formulation. Given this finding, it may be prudent to consider IVIg in management of 22qDS patients, even with normal immune evaluation, in order to decrease risks of complications associated with severe and recurrent viral respiratory infections.

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Myhre Syndrome (SMAD4 Gain of Function) Presents with Hypogammaglobulinemia and Decreased Memory T and B Cells

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Abstract/Case Report Text
Introduction: SMAD 4 is a critical downstream signaling molecule for transforming growth factor-β (TGF-β) and bone morphogenic protein (NMP1). Initially, SMAD4, also known as DPC4 Deleted in Pancreatic Cancer locus 4, was described as a tumor suppressor gene, and somatic deletion of the SMAD4 is seen in 90% of pancreatic carcinomas. Subsequently, a germline point mutation in the SMAD4 gene (p. I500V) was reported to cause Myhre syndrome (MIM#139210). Myhre syndrome is an autosomal dominant disease characterized by cognitive impairment, hearing loss, and musculoskeletal anomalies. The immunological phenotype of these patients has not been previously described, despite the critical role of TGF-β in regulating T cell response and the prevention of excessive inflammation.
Case report: A 9-year-old boy with Myhre syndrome was referred to immunology clinic for evaluation of recurrent ear infections. He developed acute otitis media infections as an infant and had tympanostomy tubes placed at one year of life. He also had a recurrent sinus infections. At two years of age, he was diagnosed with autism and sensory neuronal hearing loss. Brain MRI showed a mildly hypoplastic pituitary gland, and a thickened corpus callosum with decreased myelination. Given these findings, whole-genome sequencing was performed, which revealed a heterozygous de novo mutation in SMAD4 (p.I800V / c.1498 A>G) consistent with the diagnosis of Myhre syndrome. Through age 5, he was in the 5th percentile for height until he was started on growth hormone, which he responded to robustly. He is now he is in the 50th percentile for height. He had adenoidectomy due to sleep-disordered breathing at the age of 7 years. He is maintained on montelukast and inhaled corticosteroids for treatment of rhinitis and mild persistent asthma. He is on atenolol for the treatment of primary hypertension. On physical exam, he has facial dysmorphisms, thickened skin, and contraction of the fingers consistent with Myhre syndrome. Immunologic elevation showed significant hypogammaglobulinemia (IgG 311 mg/dL), low IgA (11 mg/dl) and normal IgM 50 mg/dl. IgG subclasses showed low IgG1 and IgG2 at 227 mg/dL and 58 mg/dL respectively. Although he was fully vaccinated, his tetanus antibody was low at 0.14 IU/ml. However, this improved after repeat vaccination to 4.38 IU/ml. Total T and B lymphocyte counts were normal; however, his memory CD4 and CD8 T cells were low for age at 2.26% and 0.5%, respectively. Additionally, his switched memory B cell count was low at 1.9%.
Conclusion: SMAD4 gain of function (Myhre syndrome) can lead to impaired memory T and B cell formation with significant hypogammaglobulinemia and low IgA. Although the patient was able to respond to protein vaccination (tetanus), it is not clear if he will be able to maintain a long-term response. In a previous study, a similar gain of

function mutation of SMAD4 has been shown to increase SMAD phosphorylation in the nucleus in fibroblasts. Further research is needed to examine the role of this mutation in T and B lymphocytes, given the interesting immunological phenotype of this patient.