INBORN ERRORS OF IMMUNITY AND PRECISION MEDICINE

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Introduction

I have no conflicts of interest
Introduction

- There are now > 400 inborn errors of immunity / primary immunodeficiency, defined by defects in T, B, NK cells or complement leading to symptoms of recurrent infections by bacteria, viruses and fungi. Additionally patients can present with immune dysregulation
  - Severe Combined Immunodeficiency (SCID)
  - Combined immunodeficiencies, less profound than SCID
  - CID with syndromic features
  - Predominant Antibody deficiencies
  - Diseases of Immune Dysregulation – presenting with autoimmunity
  - Phagocyte defects
  - Innate immune defects
  - Autoinflammatory disorders
  - Complement deficiencies
  - Bone marrow Failure
  - Phenocopies
Introduction

- Identification of the monogenic cause is of utmost importance due to:
  - Therapeutic approach
  - Targeted therapy identified in that disorder
  - Long term management
  - Prognostic awareness
  - Surveillance
  - Familial evaluation
  - Potential for a HSTC and knowns risks
Objectives

- Two cases will be presented to demonstrate
  - Presentation of PID
  - Genetic evaluation
  - Functional validation
- Highlight the importance of the immunologist and geneticist as a team
- Demonstrate how identifying the culprit gene can lead to precision therapy
Immunology Evaluation: Number and Function

- **Cellular**
  - CBC
  - Lymphocyte number
  - TREC
  - CD45 RA/RO

- **Humoral**
  - B cell panel
  - Immunoglobulin

- **Cellular**
  - T cell proliferation
  - T cell activation

- **Humoral**
  - Specific antibody titers
  - Vaccine challenge
CASE 1 - HYPEREOSINPHILIA
Full Term male with typical prenatal and postnatal history presented at 8 months of life with severe eczema, FTT and food allergy

- URTICARIAL RASHES ON HIS SKIN
- ABDOMINAL DISTENSION, CHOKING AND VOMITING
- MULTIPLE EPISODES OF WHEEZING
- 3 MRSA INFECTIONS, RECURRENT VIRAL URI, RECURRENT AOM
- PROTRUDING ABDOMEN, FTT, LAD, HSM
Warning Signs for PID in the Pediatric Population (most common)

Immune Dysregulation in Children With Down Syndrome
Additional Warning Signs:

- Severe cutaneous warts
- Autoimmune disease
- Chronic gastrointestinal disease
- Severe Eczema
- Chronic mucocutaneous candidiasis
- Dental Hygiene / Eruption of teeth
- Wound healing
Evaluation

WBC 57,000, Eosinophils 25-30% (TEC 20,000)

IgE 4,385 IU/mL

Normal T and B cell number and function

Bone marrow: increased non dysmorphic eosinophils (19%), other cell lineages and maturation wnl

Peripheral and Bone Marrow TCR and BCR rearrangement wnl

Peripheral & bone marrow hypereosinophilia mutations negative (PDGFRα-FIP1L1, JAK2, FGFRA/B, PDGFRB)

Eosinophils throughout GI tract, 40 eos/hpf in esophagus

Research WES did not report any pathogenic variants

Hyper IgE syndrome panel (STAT3, DOCK8, TKY2, SPINK5) was negative
All are AR diseases:

STAT5b is associated with AR GH insensitivity presenting with short stature and IBD.

The sequence change is a highly conserved region with a small physicochemical difference, not reported as a germline change in individuals with STAT5b condition. However, this variant has been reported to affect STAT5B protein function.
Luciferase assay demonstrated that N642H is a GOF (rajala et al., Blood 2013).

Pub med article describing “Somatic STAT5b GOF mutations in early onset nonclonal eosinophilia, urticaria, dermatitis and diarrhea” by Milner et al.

STAT5 Phosphorylation flow cytometry studies in our patient provided further validation for gain of function disease.

pSTAT studies completed by Joshua Milner at Columbia University.
Germline vs Somatic

- Custom targeted droplet digital PCR (ddPCR) in sorted leukocyte lineages determined variable fractions of cells expressing the mutant N642H.

- Mutant allele was present in 100% of eosinophils and NK cells, 75% of T cells and <50% of B cells and dendritic cells.

- This suggests a postzygotic somatic mosaicism.

- Sequencing of parents; negative for STAT5b N642H variant.

This assay was performed at the NIH.
Ruxolitinib a non selective Jak1/2 inhibitor was initiated. This is a targeted therapeutic approach in comparison to broad suppression with steroids.

Approval for non FDA indications can often be challenging.

Patient was denied despite; Prior Authorization, Peer to Peer.

Approved finally with an external NYS appeal under the indication of rare disease.

Inborn Errors of Immunity with Immune Dysregulation; From Bench to Bedside. Noterangelo et al. Fronteir in Pediatrics. 2019
Response to therapy
Future Management

- The patient went on to develop MAI abscesses of the calvarium secondary to his underlying disease or therapy complications
- Identifying his underlying genetic etiology clarifies that utility of a stem cell transplant
- He is planned for HSCT from a 10/10 matched sibling donor

**JAK inhibition in early-onset somatic, nonclonal STAT5B gain-of-function disease**

Rachel Eisenberg 1, Melissa D Gans 2, Timothy Ronan Leahy 3, Florian Gothe 4, Candice Perry 5, Mark Raffeld 5, Liqiang Xi 5, Sarah Blackstone 6, Chi Ma 6, Sophie Hambleton 7, Joshua D Milner 8
Lessons

The diagnosis may be hidden among your “VUS”

Somatic variant can have an early onset presentation

Proof of somatic versus germline variants involves sequencing from different cells

A luciferase assay is used to prove a gain of function

Reaching out to research based colleagues is of utmost importance

Hard work pays off when you can now offer targeted therapy to your patient

Offering a HSCT would not be an option without confirming his diagnosis
CASE 2 – TRISOMY 21 AND MORE
Presentation

- 10 month old ex 34 week baby boy was diagnosed with Trisomy 21 in utero via CVS.
- NBS results showed a low TREC associated with Severe Combined immunodeficiency (SCID).
- SCID is a disease defined by absent T cells with early onset severe infections (viral and opportunistic), fatal within the first 1-2 years of life without a HSCT.
- SCID was ruled out in our patient based on normal T cell function and presence of >200 CD45RA T cells.
- T cell lymphopenia was felt to be secondary to T21, a common non SCID cause of T cell lymphopenia.
## Non SCID causes of Abnormal TREC

<table>
<thead>
<tr>
<th>Conditions</th>
<th>%</th>
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<tbody>
<tr>
<td><strong>Multisyndromes with variable T-cell deficiency</strong></td>
<td></td>
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<tr>
<td>DiGeorge/chromosome 22q11.2 deletion</td>
<td>57</td>
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<tr>
<td>Trisomy 21</td>
<td>15</td>
</tr>
<tr>
<td>Ataxia telangiectasia</td>
<td>3</td>
</tr>
<tr>
<td>CHARGE syndrome</td>
<td>2</td>
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<tr>
<td><strong>Secondary T lymphopenia</strong></td>
<td></td>
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<tr>
<td>Congenital cardiac anomalies</td>
<td>25</td>
</tr>
<tr>
<td>Other congenital anomalies</td>
<td>38</td>
</tr>
<tr>
<td>Vascular leakage, third spacing, hydrops</td>
<td>13</td>
</tr>
<tr>
<td>Neonatal leukemia</td>
<td>3</td>
</tr>
<tr>
<td>Maternal immunosuppressive medications</td>
<td>3–5</td>
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<td>Extreme preterm birth (T cells become normal over time)</td>
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</table>

**Idiopathic T lymphopenia (no gene defect, few naïve cells, impaired T cell function)**
Presentation

- WBC 167K; diagnosed with Transient Abnormal Myelopoiesis treated with cytarabine
- Persistent Lymphopenia (CD3 <1000 cells)
- Repeated serratia and MRSA tracheitis
- Findings consistent with a hyperinflammatory state, HLH/MAS
Lab Findings and Clinical findings:

- Prolonged Fevers
- Lymphopenia and Anemia (Hb 6.3)
- Waxing and waning neutropenia
- Mild splenomegaly
- Cervical Lymphadenopathy
  - No evidence of hemophagocytosis
- Basic immunology workup revealed lymphopenia, normal % of T and B cell, intact T cell function, elevated IgG, normal IgA and IgM, protective antibody titers

<table>
<thead>
<tr>
<th>Lab</th>
<th>Results (ref range)</th>
</tr>
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<tbody>
<tr>
<td>SIL2</td>
<td>2,521 (398-1940) U/mL</td>
</tr>
<tr>
<td>LFT</td>
<td>wnl</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>70 ( &lt;100)</td>
</tr>
<tr>
<td>Ferritin</td>
<td>6,000 (25-270) ng/mL</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>219 (187-502) mg/dL</td>
</tr>
<tr>
<td>IL18</td>
<td>148,566 (89-540) pg/mL</td>
</tr>
<tr>
<td>S100A12</td>
<td>26,911 (32-385) ng/mL</td>
</tr>
<tr>
<td>CXCL9</td>
<td>161 (&lt;121) pg/mL</td>
</tr>
<tr>
<td>CD107a</td>
<td>qns</td>
</tr>
<tr>
<td>Perforin flow</td>
<td>Normal</td>
</tr>
<tr>
<td>XLP1/2 by Flow</td>
<td>SAP and XIAP present</td>
</tr>
</tbody>
</table>
Table 2. List of diagnostic criteria for HLH that were used in the Histiocyte Society HLH-2004 study

(A) Genetic defect consistent with HLH or (B) 5 out of 8 clinical and laboratory criteria fulfilled

- Fever
- Splenomegaly
- Cytopenia in ≥2 cell lineages
  - Hemoglobin <9 g/dL, in neonates <10 g/dL
  - Platelet count <100 × 10³/mL
  - Neutrophil count <1 × 10³/mL
- Hypertriglyceridemia (>265 mg/dL) or hypofibrinogenemia (<150 mg/dL)
- Hyperferritinemia (>500 ng/mL)
- Soluble CD25 >2400 U/mL (or elevated compared with laboratory-defined normal ranges)
- Hemophagocytosis in bone marrow, spleen, lymph nodes, or liver
- Low or absent NK-cell cytotoxicity
Questions?

Is his lymphopenia and presentation beyond what is seen in T21?

Are his infections iatrogenic or a clue to an underlying PID?

Does he also have a primary HLH?

Is it time for further genetic evaluation despite his known diagnosis?
Referral to Immunogenetics Clinic

Primary Immunodeficiency Panel (407) to evaluate for PID associated with HLH like presentation

Copy number gains were identified for the AIRE, IFNGR2, IL10RB, ITGB2 gene(s) on chromosome 21, consistent with T21 diagnosis

However; IFNGR1 gain of entire coding sequence is NOT on chromosome 21
Microarray Results

Arr[hg19]: 6q23.3(136,709,632-137,979,598)X3 and (21)X3

1.13kb duplication on 6q including the following genes: MAP7, MAP3K5, LOC101928461, LOC101928429, PEX7, SLC35D3, NHEG1, IL22RA2, IFNGR1

Duplication on chromosome 6 was maternally inherited
What is Interferon?

- Interferons are cytokines with potent inflammatory and antiviral function.
- Engagement of interferon with its receptor leads to phosphorylation of JAK STAT pathways and downstream transcription of hundreds of IFN stimulated genes.
- There are 6 interferon receptors:
  - Chromosome 21 - IFNAR1, IFNAR2, IFNGR2, IL-10R2
  - Chromosome 6 - IFNGR1
  - Chromosome 1 - IL12R1

![Diagram of interferon signaling pathways](image-url)
INTERFERON DISEASE

- Too Little $\rightarrow$ Increased susceptibility to viral agents
- Too much $\rightarrow$ cognitive defects and skin inflammation

Down Syndrome and Type I interferon; not so simple. Malle and Bogunovic. 2021
Interferonopathy and Trisomy 21

There are three copies of IFNAR1, IFNAR2 and IFNGR2 in T21 patients

It is well established that:

1. T21 patients have higher expression of IFNAR1 and IFNAR2 on their cells

2. Cells from individuals with T21 have basal IFNg signaling and hyper respond to type I IFN

3. T21 patients have signs of inflammation in the absence of any detectable infection, indicative of a proinflammatory cellular state

Down Syndrome and Type I interferon; not so simple. Malle and Bogunovic. 2021
Down Syndrome and Type I interferon; not so simple. Malle and Bogunovic. 2021
T21 and Cytokine Storm – Espinosa et al

The IFN response, key for initiating and amplifying a cytokine storm is much more active in patients with T21

Down Syndrome and COVID-19: A Perfect Storm? Espinosa et al
The inflammasome and T21

- The inflammasome is a multi-protein complex that generates IL1 family cytokines
  - IL1b and IL18

- Children with DS have significantly elevated levels of IL-1b

- Increased Inflammasome activation can lead to symptoms of hyperinflammation

- There is a paucity of research regarding the inflammasome and T21
The patient has T21 with three copies of IFNAR1, IFNAR2 and IFNGR2

Additionally he has a duplications on chromosome 6 encompassing IFNGR1

Lastly the patients IL-18 is extremelly elevated

His presentation is that of hyperinflammation / HLH like, cytokine storm like
Hypothesis regarding the patient

Hyperinflammatory state is secondary to overexpression of 5 out of 6 of the IFNR genes and elevated inflammasome cytokines seen in T21

Immune Dysregulation in Children With Down Syndrom
Management

• JAK inhibition downstream of IFN Receptors has been successfully used to treated IFN mediated disease and would be a targeted approach
  • Cases reports and clinical trials using JAK inhibition to treat alopecia associated with T21
• Anakinra is used to target the inflammasome pathway and is used in rheumatologic causes of MAS in contrast to primary or acquired HLH causes
Management

- Lab results were consistent with a pattern seen in rhematologic causes of MAS vs primary of acquired HLH.
- The patient was therefore started on Anakinra with rapid response:
  - Afebrile
  - Ferritin normalized
  - S1L2 normalized
  - IL18 decreased from 150,000 to 20,000
  - Anemia resolved
  - Lymphadenopathy resolved
- He continues this therapy for nearing one year, dose was initially decreased (4mg/kg/day to 2mg/kg/day)


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Conclusions of Case 2

- PID should be considered in children with classic warning signs of PID
- PID should also be evaluated in patients presenting with hyperinflammatory states or signs of immunedysregulation
- T21 is considered an interferonopathy with manifestation of immune dysregulation
  - Increased viral infections
  - Autoimmune skin conditions
  - Hyper inflammatory states
TABLE 1 | Targeted therapies used in disorders of immunodysregulation and hyperinflammation.

<table>
<thead>
<tr>
<th>Molecular Target</th>
<th>Molecular Structure</th>
<th>Drug</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD82</td>
<td>mAb</td>
<td>Alemtuzumab</td>
<td>Hemophagocytic lymphohistiocytosis</td>
</tr>
<tr>
<td>JAK</td>
<td>Small molecule inhibitor</td>
<td>Ruxolitinib</td>
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</tr>
<tr>
<td>IFN-γ</td>
<td>mAb</td>
<td>Eminapuzumab</td>
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<tr>
<td>mTORC3</td>
<td>Macrolide compound</td>
<td>Sirolimus</td>
<td>NLRP4-GOF, POMP deficiency, CTLA-4 haploinsufficiency, APDS</td>
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<tr>
<td>B7-1 (CD80)</td>
<td>CTLA-4 IgG fusion protein</td>
<td>Abatacept</td>
<td>CTLA-4 haploinsufficiency, LRBA deficiency</td>
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<tr>
<td>B7-2 (CD86)</td>
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<td>Belatacept</td>
<td>CTLA-4 haploinsufficiency, LRBA deficiency</td>
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<tr>
<td>IL-1R</td>
<td>Recombinant human IL-1R antagonist</td>
<td>Anakinra</td>
<td>Cryopyrin-associated periodic fever syndromes</td>
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<tr>
<td>IL-1β</td>
<td>Anti-human IL-1β IgG mAb</td>
<td>Canakinumab</td>
<td>CAPS</td>
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<tr>
<td>IL-10</td>
<td>IgG1x recombinant humanized mAb</td>
<td>Tolizeumab</td>
<td>STAT3-GOF, APEM, IRIS, BPA</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Fusion protein</td>
<td>Etanercept</td>
<td>SAVI</td>
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<tr>
<td></td>
<td>Chimeric mAb</td>
<td>Infliximab</td>
<td>CANDLE syndrome, POMP deficiency</td>
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<tr>
<td></td>
<td>Humanized mAb</td>
<td>Adalimumab</td>
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<tr>
<td>JAK1 and JAK2</td>
<td>Small molecule inhibitor</td>
<td>Ruxolitinib</td>
<td>STAT3-GOF, BACO</td>
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<td></td>
<td></td>
<td>Brolucizumab</td>
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<tr>
<td>JAK1 and JAK3</td>
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<td>Tolulitinib</td>
<td>CANDLE syndrome</td>
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<td>P110κ</td>
<td>Lenalidomide</td>
<td>Lenalidomide</td>
<td>APDS</td>
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<tr>
<td>IL-18 binding protein</td>
<td>Recombinant IL-18 binding protein</td>
<td>Takedaing-α</td>
<td>NLRP4-GOF, APDS</td>
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<td>B-lymphocyte stimulator</td>
<td>Human mAb IgG1 k</td>
<td>Belemumab</td>
<td>Autoimmune cytopenias</td>
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<td>Plasma cells</td>
<td>Proteasome inhibitor</td>
<td>Bortezomib</td>
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<tr>
<td></td>
<td>Human mAb</td>
<td>Daratumumab</td>
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</table>
Questions?

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