McMaster University
Department of Pediatrics
3A-Health Sciences Centre
1280 Main Street West, Hamilton ON L8S 4K1
Tel: 905.521.2100
McMaster Children's Hospital
HAMILTON HEALTH SCIENCES

Welcome to the Problem-Based Learning (PBL) Cases for Basic Immunology!

These cases were designed to provide learners with an interactive approach to understanding the fundamentals of basic and clinical immunology through Problem-Based Learning (PBL). Each case presents real-world scenarios that challenge you to take a deep dive into the immune system pathways. Below each case you will find guiding questions and recommended resources.

For educators: Please contact Dr. Jenny Garkaby by email if you have any questions or if you develop a new case that you would like to contribute for inclusion. Please do not hesitate to submit feedback to garkabyj@mcmaster.ca

This learning tool was developed through the collaborative efforts of Drs:
Jenny Garkaby
Rae Brager
David Fahmy
Anahita Dehmoobad Sharifabadi
Vivian Szeto
Andrew Wong-Pack

How to approach a PBL case effectively?

On the first tutorial:

1. Assign roles, if applicable:

Facilitator: Guides the discussion and keeps the group on track.
Scribe (optional): Takes notes and summarizes key points.
Timekeeper: Ensures the group stays within the allotted time.

2. Read the case aloud as a group
3. Identify the main clinical problem and clarify unfamiliar terms and concepts
4. Brainstorm potential differential diagnoses based on the presenting problem
5. Discuss what knowledge gaps exist
6. Create a list of topics to research further
7. Assign topics to individuals

On the following tutorial:

1. Discuss key clinical findings and laboratory data
2. Review the relevant immune pathway and how a defect in this pathway explains the clinical features
3. Consider evidence-based guidelines and literature to support decision-making
4. Summarize key takeaways as a group
5. Answer practice questions

End of tutorial feedback: Reflect on what worked well and what could be improved for future sessions.

ENJOY IMMUNOLOGY!

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Complement system

Recurrent Leukocytoclastic Vasculitis and Complement Deficiency in an Adolescent Male
This case aims to provide a comprehensive understanding of the immunological pathways and clinical aspects of complement deficiency.

Cole Complemento is a 16-year-old male born to consanguineous parents who presented with a one-year history of recurrent leukocytoclastic vasculitis confirmed on skin biopsy. His medical history includes recurrent otitis media during his childhood and adolescence, which required myringotomy tubes at the age of 12 years. There was one episode of meningitis with strep. Pneumoniae in early childhood with no clinical sequelae. Family history reveals multiple loops of consanguinity and 3 first cousins with systemic lupus erythematosus (SLE) nephritis, of whom one passed away due to SLE complications.
Laboratory investigations show normal CBC, normal kidney function, no proteinuria, normal immunoglobulins level, normal lymphocyte subsets with no T or B cell lymphopenia and normal vaccine titers. ANA, cANCA, pANCA were negative, however, RF was positive. CRP was not elevated.
Complement testing revealed a normal C4 at 19 mg/dL (range 13-39 mg/dL). C3 levels were undetectable at <29 mcg/dL (reference range 81-157 mcg/dL) and CH50 level was also undetectable at <14U/ mL, supporting a diagnosis of clinical C3 deficiency.
Genetic testing identified a homozygous missense mutation in the C3 gene.

Questions:

1. Explain the role of the complement system in innate immunity
2. How are complement proteins activated in response to pathogens?
3. What are the three complement activation pathways and their triggers?
4. How are complement proteins being regulated?
5. Why is there autoimmunity in patients with complement deficiency?
6. What diagnostic tests and therapeutic interventions are available for managing complement deficiencies?
7. Identify common clinical manifestations associated with C3 deficiency and suggest treatment.
8. What is the CH50 test?

Resources:

1. Janeway's Immunobiology
2. Basic Immunology, Abul K Abbas
3. Erdei A, Fust G, Gergely J. The role of C3 in the immune response. Immunol Today 1991;12:332–7. https://doi.org/10.1016/0167-5699(91)90011-H
4. Schröder-Braunstein J, Kirschfink M. Complement deficiencies and dysregulation: Pathophysiological consequences, modern analysis, and clinical management. Molecular Immunology 2019;114:299-311 https://doi.org/10.1016/j.molimm.2019.08.002 .
5. Brodszki, N et al. European Society for Immunodeficiencies (ESID) and European Reference Network on Rare Primary Immunodeficiency, Autoinflammatory and Autoimmune Diseases (ERN RITA) Complement Guideline: Deficiencies, Diagnosis, and Management. J Clin Immunol 40, 576–591 (2020). https://doi.org/10.1007/s10875-020-00754-1
6. https://www.youtube.com/watch?v=zSZrXeEj35I

7. As of minute 25: https://www.youtube.com/watch?v=JGyUoXG_6C0
8. https://www.youtube.com/watch?v=Uc4nq4Lazo4&t=370s

A Young Child with Recurrent Neisseria Infections

Lucas Meyer, a 7-year-old boy, is referred to Pediatric Infectious Diseases for evaluation of recurrent Neisseria infections.

Lucas has had two episodes of meningococcal meningitis. The first episode occurred at age 6, presenting with fever, headache, neck stiffness, and a petechial rash. He was hospitalized and treated with IV antibiotics. The second episode happened 6 months ago with similar symptoms, successfully treated again. Between episodes, he has been well with no other infections. He is described to be generally healthy and fully vaccinated (including meningococcal vaccines). No history of recurrent bacterial infections other than Neisseria. No significant hospitalizations besides meningitis. No known immunodeficiencies in the family, however, they are from the same remote area in Ontario. No consanguinity. A paternal uncle died suddenly from meningitis at age 20.

Physical Exam:

Well-appearing today, no rash or focal signs.Vitals are normal, no fever. Normal growth parameters. No lymphadenopathy or hepatosplenomegaly.

Investigations

CBC: Normal WBC and differential. Immunoglobulins: Normal IgG, IgA, IgM levels and normal vaccine titres. Total hemolytic complement activity (CH50) is undetectable. C3 and C4: Normal. Specific complement component assay: C8 deficiency confirmed (absent C8 protein).

Neisseria species isolated: Neisseria meningitidis serogroup B.

Questions:

1. Suggest a management plan for this patient
2. What is the function of the terminal complement components, especially C8, in bacterial killing?
3. Why do patients with C8 deficiency have increased susceptibility specifically to Neisseria species?
4. How is complement activity measured, and what do CH50 and AH50 tests tell us?
5. How does vaccination protect patients with complement deficiencies? Are vaccines sufficient?
6. What is the genetic inheritance pattern of C8 deficiency, and what implications does this have for family members?

Resources:

1. Janeway's Immunobiology
2. Basic Immunology, Abul K Abbas
3. Erdei A, Fust G, Gergely J. The role of C3 in the immune response. Immunol Today 1991;12:332–7. https://doi.org/10.1016/0167-5699(91)90011-H

4. Schröder-Braunstein J, Kirschfink M. Complement deficiencies and dysregulation: Pathophysiological consequences, modern analysis, and clinical management. Molecular Immunology 2019;114:299-311 https://doi.org/10.1016/j.molimm.2019.08.002 .
5. Brodszki, N et al. European Society for Immunodeficiencies (ESID) and European Reference Network on Rare Primary Immunodeficiency, Autoinflammatory and Autoimmune Diseases (ERN RITA) Complement Guideline: Deficiencies, Diagnosis, and Management. J Clin Immunol 40, 576–591 (2020). https://doi.org/10.1007/s10875-020-00754-1
6. https://www.youtube.com/watch?v=zSZrXeEj35I
7. As of minute 25: https://www.youtube.com/watch?v=JGyUoXG_6C0
8. https://www.youtube.com/watch?v=Uc4nq4Lazo4&t=370s

Innate Immune system

Recurrent HSV encephalitis and Toll-like receptor defect

This case aims to review the role of Toll-like receptors in innate immunity and viral recognition.

Ahmed Abd al-Rahman is a previously healthy, fully vaccinated, and developmentally appropriate 16-month-old boy who presented to the pediatric emergency department (ED) with fever, vomiting, static gaze, unresponsiveness, and sporadic flexing of his arms lasting about 45 minutes. Upon arrival, he received midazolam by EMS with clinical improvement and was further managed in the ED before admission to the pediatric ward.

The following day, Ahmed continued to experience seizures despite treatment with midazolam, lorazepam, and leviteracatam. His EEG revealed persistent subclinical seizures. Antimicrobials and IV acyclovir were initiated empirically due to suspicion of viral encephalitis. Despite completing a course of acyclovir as per infectious disease recommendations, his seizure burden fluctuated and worsened again. His lumbar puncture initially showed 43 leukocytes, glucose of 3.5 mmol/L, and protein of 0.22 g/L, with a positive result for HSV1 in the cerebrospinal fluid (CSF). Subsequent LPs showed persistence of HSV1 in the CSF.

Ahmed's brain MRI indicated findings suggestive of meningoencephalitis with bilateral symmetrical involvement of cortical and subcortical areas in all cerebral lobes. Due to the severity of his illness, lack of improvement, and prolonged disease course, the immunology team was consulted to investigate possible underlying immunodeficiency.

Ahmed was born at term via elective C-section due to parental preference. His parents are first cousins of Pakistani descent, and this was his mother's first pregnancy. Pregnancy and birth were otherwise unremarkable.

On physical examination, Ahmed exhibited equal and reactive pupils, but did not react to light stimulus bilaterally. He demonstrated an appropriate gag reflex when an NG tube was inserted, and symmetric facial movements. He did not open his eyes spontaneously, however he moved all limbs spontaneously with low peripheral tone. There was no spasticity or clonus at the ankles, and he responded weakly to tactile stimulus in the extremities with withdrawal.

Initial investigations including complete blood count, flow cytometry for B and T cells, and quantitative immunoglobulin levels were normal, as were vaccine titers. However, a primary immunodeficiency panel revealed a homozygous pathogenic variant in the TLR3 gene.

Questions:

1. Describe the immune response in a patient with a viral infection.
2. What are the key differences between innate and adaptive immunity?
3. Name the key players in innate immunity (cells, pattern recognition receptors, TLRs)
4. How do the innate and adaptive immune responses collaborate to eliminate pathogens? (Through which cell?)
5. Context to other pathways: Which pathways are induced by TLR stimulation?
6. Review the different ligands of the TLRs
7. Discuss management strategies for patients with TLR3 deficiency, including antimicrobial therapy and supportive care.

Resources:

1. Janeway's Immunobiology
2. Basic Immunology, Abul K Abbas
3. Lim HK et al. TLR3 deficiency in herpes simplex encephalitis: high allelic heterogeneity and recurrence risk. Neurology. 2014. doi: 10.1212/WNL.0000000000000999
4. https://www.youtube.com/watch?v=ZwxAWx_y0Zw

Innate immune system: The Inflammasome

The role of the inflammasome in cold-triggered inflammation
This case aims to provide a comprehensive understanding of the inflammasome activation pathways and clinical aspects of defects in the inflammasome signalling

Emma Frost, a 24-year-old woman, presents to her primary care physician with a history of recurrent episodes of fever, rash, joint pain, and malaise. She reports that these episodes typically occur shortly after exposure to cold temperatures and resolve during the summer. The symptoms last for several hours to a few days and resolve spontaneously. Emma has noticed that the episodes have become more frequent over the past year. During her episodes, Emma's temperature rises to 39°C, and she develops a diffuse erythematous rash over her extremities. She experiences severe joint pain, especially in her elbows and knees, which limits her mobility during these episodes. Laboratory tests during an episode show elevated leukocytes, C-reactive protein and erythrocyte sedimentation rate with no evidence of inflammation when she is well.
Emma's medical history is otherwise unremarkable. She reports no family history of similar symptoms.

During an episode, Emma appears febrile and uncomfortable. Her skin shows diffuse erythema over her arms and legs. There is no evidence of joint swelling, however her elbows and knees were sensitive.

Genetic testing shows a pathogenic variant in the NLRP3 gene, confirming a diagnosis of Familial Cold Autoinflammatory Syndrome.

Questions:

1. Describe the key components of an inflammasome and how it is activated (Sensor proteins, what are CARD and caspase or other components?)
2. What are the pro-inflammatory cytokines secreted after inflammasome activation and what is their downstream effect.
3. Explain the mechanism of IL-1β production and its role in the inflammatory response.
4. What are the potential therapeutic strategies targeting inflammasomes in disease?
5. Explore treatment options for FCAS, including IL-1β blockade with medications like Anakinra
6. What do you expect to see on a skin biopsy from that patient and why?

Resources:

1. Janeway's Immunobiology
2. Basic Immunology, Abul K Abbas
3. Rathinam VA et al. Inflammasome Complexes: Emerging Mechanisms and Effector Functions. Cell. 2016 May 5;165(4):792-800. doi: 10.1016/j.cell.2016.03.046
4. https://www.youtube.com/watch?v=l4-DYqS_LbY&t=49s
5. Putnam CD et al. The discovery of NLRP3 and its function in cryopyrin-associated periodic syndromes and innate immunity. Immunol Rev. 2024 doi: 10.1111/imr.13292.

Neutrophil function

This case illustrates how defects in phagocyte oxidative burst impair microbial killing, leading to recurrent infections and highlights the role of reactive oxygen species in host defence.

Neil Trophil, a 7-year-old boy, was referred to the immunology clinic for possible immunodeficiency due to a recent admission for a deep-seated infection in form of a liver abscess.

He was born at term following a normal pregnancy and delivery to non-consanguineous parents of European origin. His family history is significant for discoid lupus in Neil's mother. His father and older sister are both healthy.

At 9 months old, Neil was admitted to the hospital with severe pneumonia. Imaging revealed a large lung abscess, requiring chest tube drainage. Cultures grew Staphylococcus aureus. He received prolonged antibiotic therapy and eventually recovered. Neil experienced recurrent respiratory infections, which were initially attributed to his early severe pneumonia.

At 6 years, he was diagnosed with inflammatory bowel disease after presenting with chronic diarrhea, perianal abscesses, abdominal pain, and slow weight gain. Colonoscopy confirmed the diagnosis of Crohn's disease by pathology with multiple granulomas on his GI biopsy. He was started on anti-TNF

therapy (infliximab) with some improvement in his symptoms. Despite his IBD being better controlled, Neil presented with fever, fatigue, nausea and abdominal pain. Abdominal US confirmed a deep-seated liver abscess. Cultures of the liver abscess grew Burkholderia cepacia.

On physical Exam, Neil appeared pale and thin, with mild hepatosplenomegaly. There were no other significant findings.

Laboratory Results revealed normal white blood cell count with mild anemia and elevated ESR and CRP, T, B, and NK cell quantitation were normal, IgG was elevated at 15 gr/l with normal IgM and IgA levels.

Neutrophil oxidative burst index is provided below with the patient compared to a healthy control.

Genetic Testing confirmed a mutation in the CYBB gene, consistent with X-linked Chronic Granulomatous Disease.

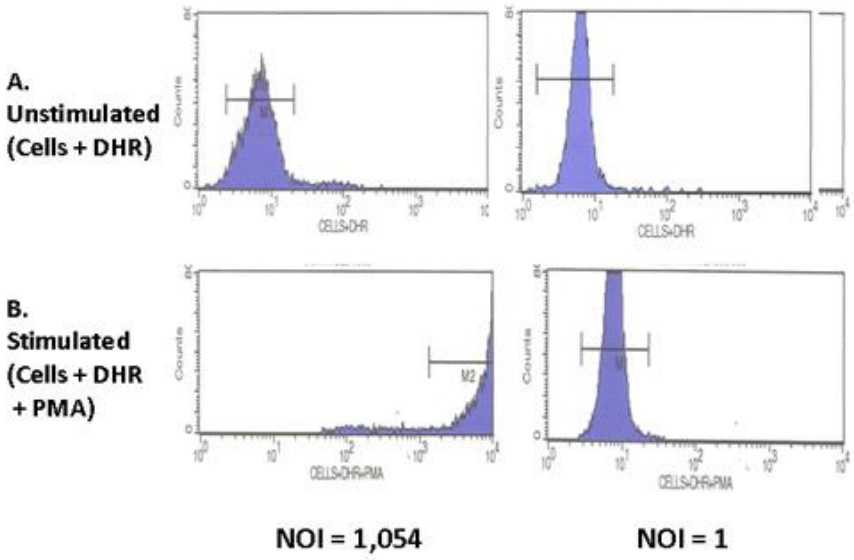

Figure source: https://www.cytometry.org/newsletters/eICCS-2-1/article6.php

Questions:

1. What are the key functions of neutrophils in combating infections?
2. Describe the recruitment of neutrophils to the inflammation site, what are the cytokines that mediate this and how can it be targeted with a biologic medication?
3. Explain the process of phagocytosis and the importance of the oxidative burst in killing pathogens.
4. Explain how the defects in the NADPH oxidase complex result in impaired neutrophil function.
5. What are the typical infectious agents seen in patients with CGD?
6. What are the potential risks of using anti-TNF therapy in patients with CGD and why?
7. What do you expect to see on flow cytometry if you send a NOBI on this patient's mother?

Resources:

1. Janeway's Immunobiology
2. Basic Immunology, Abul K Abbas
3. Zerbe CS et al. Functional neutrophil disorders: Chronic granulomatous disease and beyond. Immunol Rev. 2024 Mar;322(1):71-80. doi: 10.1111/imr.13308.
4. The DDx of S. Aureus infection: Kurz H et al. Inborn errors of immunity with susceptibility to S. aureus infections. Front Pediatr. 2024 doi: 10.3389/fped.2024.1389650.
5. https://www.youtube.com/watch?v=8x-9NLmE2qY
6. https://www.youtube.com/watch?v=otdkIjfBkrQ

Amira El-Khatib — A Neonate with Delayed Cord Separation and Recurrent Infections

Amira El-Khatib is a 9-week-old female infant who has been referred to the Pediatric Immunology team due to persistent umbilical inflammation, delayed separation of the umbilical cord, and concerns about impaired wound healing.
She was born at term following an uncomplicated pregnancy and spontaneous vaginal delivery. Her Apgar scores were reassuring and she was discharged home with her parents at 36 hours of life.
Amira is the first child of healthy, consanguineous parents of Middle Eastern descent.
The family history is unremarkable for early infant deaths, recurrent infections, or known immunodeficiencies.

Her clinical course began to raise concerns at around two weeks of life, when the umbilical stump remained attached and began to show signs of erythema and purulent discharge. Despite a course of oral antibiotics, the umbilical stump did not separate until 55 days of life, and the periumbilical area continued to appear inflamed. Around the same time, Amira developed a localized infection at the site of a venipuncture, with redness and swelling but no apparent pus formation. She was admitted briefly to hospital for IV antibiotics and improved clinically.

On examination, Amira appears well and alert. Her weight and length are appropriate for age. Her umbilicus shows mild erythema with residual granulation tissue but no active discharge. A small area of healing cellulitis is noted on her right forearm where an IV line had been placed.
There is no hepatosplenomegaly or lymphadenopathy. Her skin is otherwise clear, and there are no oral lesions or signs of systemic illness.

Initial laboratory investigations reveal a markedly elevated white blood cell count of 48 $x10^9$/L, with an absolute neutrophil count of 38 $x10^9$/L. C-reactive protein was mildly elevated.
Immunoglobulin levels, including IgG, IgA, and IgM, are within normal limits for age.
Flow cytometry of her lymphocytes was normal. Flow cytometry of peripheral blood leukocytes revealed an absence of CD18 expression on neutrophils. CD11 expression is also absent.
Her neutrophil oxidative burst index test is performed and is normal, ruling out chronic granulomatous disease.

Based on the constellation of delayed umbilical cord separation, high neutrophil counts, poor pus formation despite bacterial infections, and the absence of CD18 integrin on neutrophils, a diagnosis of leukocyte adhesion deficiency type I is made. Genetic confirmation of the diagnosis is underway.

Questions:

1. The family is counselled about the nature of the disorder and its implications. What does the consultation include?
2. What is the normal function of the CD18 integrin, and how does its absence impair neutrophil function?
3. Why do patients with LAD have high neutrophil counts but impaired infection control?
4. How do you distinguish LAD from CGD based on clinical signs and lab testing?
5. Why is pus formation absent in LAD even during bacterial infections?
6. What are the indications for HSCT in LAD, and what factors influence its timing and success?

NK cell function

This case aims to review impaired NK cell function. HLH is a life-threatening condition characterized by excessive immune activation and cytokine release due to NK dysfunction.

Mira is a 5-month-old female infant who presented with a history of persistent fevers, low oral intake and irritability. She was seen by her family doctor on the 5th day of her fever and was found to have hepatosplenomegaly on physical exam which was attributed to viral illness. It is worth noting that her family history is remarkable for consanguinity. Her mom mentioned having a cousin who passed away in India for an unknown reason at a young age and is very concerned about Mira's fevers. They decide to go to the ED for additional work up.

At the ED, Mira appeared febrile and irritable on examination. Physical examination confirmed hepatomegaly and splenomegaly. There were no apparent signs of respiratory distress or neurological deficits and no skin rash.

Laboratory investigations revealed significant abnormalities: CBC showed hemoglobin of 90 g/L, platelet count of 80 x 10^9/L (per liter), and white blood cell count of 3.2 x 10^9/L with absolute neutrophil count of 0.3 x 10^9/L. Liver function tests demonstrated elevated levels of total bilirubin, ALT, and AST up to ~500 U/L. Ferritin level was markedly elevated at 4,800 µg/L, and triglyceride levels were elevated at 250 mg/dL. CRP was elevated however ESR remained normal. Hemophagocytic Lymphohistiocytosis (HLH) was suspected based on clinical and laboratory findings. On the following day she underwent a Bone marrow aspirate and biopsy which showed a hypercellular marrow with prominent histiocytes and evidence of hemophagocytosis.

NK degranulation assay, which revealed impaired NK cell activity suggestive of an underlying genetic defect affecting cytotoxic function. Genetic testing for familial HLH mutations was planned due to consanguineous parental background.

Questions:

1. How do NK cells perform their effector function (i.e. how do they kill?); How are they triggered and activated?
2. Describe the production and function of IFN-γ
3. Describe NK cell receptors and how they function
4. Name classes of NK cell activating and inhibitory receptors

5. What are innate-like lymphocytes (ILC)? What are the main classes of ILCs? Where are they found?
6. What is the DDx from a genetics perspective for this patient's HLH?

Resources:

1. Janeway's Immunobiology
2. Basic Immunology, Abul K Abbas
3. https://www.youtube.com/watch?v=fTjrM0zFgq8&t=1704s
4. https://www.youtube.com/watch?v=ZCRYq8h60xk
5. Verbsky, J. W et al (2006). Hemophagocytic lymphohistiocytosis: Diagnosis, pathophysiology, treatment, and future perspectives. Annals of Medicine 2006. https://doi.org/10.1080/07853890500465189

NK deficiency: A Teenager with Recurrent Infections and Hematologic Abnormalities

Muhammad is a 14-year-old boy recently immigrated from Pakistan, presents to your Pediatric Immunology clinic for evaluation of suspected primary immunodeficiency. He has a history of recurrent pneumonias and otitis media, asthma, hepatosplenomegaly, and left eye blindness diagnosed in infancy. His family history is notable for consanguinity and a sister who died of complications following a bone marrow transplant for thalassemia major. No known history of immunodeficiency or malignancy in the family.

- What are your initial thoughts?
- What further information would you need?
- What are the warning signs of primary immunodeficiency in this case?

You review his history and learn the following:

- Recurrent pneumonias since childhood (4–5 lifetime).
- History of type 3 von Willebrand disease and thalassemia minor.
- Past NICU admission at birth for presumed lung infection.
- Recurrent otitis media (no recent infections).
- Left eye blindness of unclear cause.

Physical exam was remarkable for splenomegaly

Imaging shows hepatosplenomegaly. He is up-to-date on some vaccines, but titers show no protective immunity to diphtheria or tetanus despite recent vaccination.
Lab investigations reveal:

- low IgG level at 3.40 g/L (normal: 5.81–16.52), while his IgA was 0.93 g/L and IgM was 0.98 g/L—both within normal limits. His IgE was at 2 KIU/L.
- On flow cytometry, his absolute CD3+ T cell count was elevated at 2.62 $\times 10^9$/L. CD4+ T helper cells were 0.99 $\times 10^9$/L and CD8+ cytotoxic T cells were 0.76 $\times 10^9$/L. His CD4/CD8 ratio was 1.3. B cells (CD19+) were elevated at 0.81 $\times 10^9$/L. Natural Killer

(CD56+) cells **were notably low at 0.04 ×10⁹/L, just barely above the lower limit of detection, with a proportion of only 1%**

- Absent responses to diphtheria and tetanus serology
- Genetic testing revealed that Muhammad Aaqil Khan is hemizygous for a variant in the IL2RG gene, specifically c.-105C>T, located in the 5' untranslated region. This variant has not been reported in population databases such as gnomAD. It has, however, been described in the literature in two unrelated males with **mild or atypical combined immunodeficiency** (Chandra et al., 2016 [PMID: 26525228]; Firtina et al., 2020 [PMID: 32445296]).

Questions:

1. How do you classify this patient's immunodeficiency?
2. Suggest management, what factors determine initiation of IVIG in this patient?
3. What is the function of the common gamma chain (γc) in the immune system, and which cytokines require it? Focus on their roles in T, B, and NK cell development and function.
4. How can a patient with an IL2RG variant still have normal or even elevated T and B cell counts? Explore the idea of hypomorphic or leaky mutations
5. Why are NK cells decreased in IL2RG-related immune deficiencies?
6. What functional assays could help clarify whether this 5' UTR variant is pathogenic?
7. How would your management differ if this variant were definitively shown to impair IL2RG expression or function?

References:

1. X-Linked Severe Combined Immunodeficiency. Allenspach EJ, Rawlings DJ, Petrovic A, et al GeneReviews. Updated 2021 Aug 5.
2. The Common Cytokine Receptor Γ Chain Family of Cytokines. Lin JX, Leonard WJ. Cold Spring Harbor Perspectives in Biology. 2018;10(9):a028449.
3. The Interleukin-2 Receptor Gamma Chain: Its Role in the Multiple Cytokine Receptor Complexes and T Cell Development in XSCID. Sugamura K, Asao H, Kondo M, et al. Annual Review of Immunology. 1996;14:179-205. doi:10.1146/annurev.immunol.14.1.179.
4. Functional Insights of an Uncommon Hypomorphic Variant in IL2RG as a Monogenic Cause of CVID-like Disease With Antibody Deficiency and T CD4 Lymphopenia. González-Torbay A, Reche-Yebra K, Clemente-Bernal Á, et al. Frontiers in Immunology. 2025;16:1544863. doi:10.3389/fimmu.2025.1544863.
5. Partial T Cell Defects and Expanded CD56 NK Cells in an SCID Patient Carrying Hypomorphic Mutation in the IL2RG Gene. Cifaldi C, Cotugno N, Di Cesare S, et al. Journal of Leukocyte Biology. 2020;108(2):739-748. doi:10.1002/JLB.5MA0220-239R. ding Journal
6. Clean Up by Aisle 2: Roles for IL-2 Receptors in Host Defense and Tolerance. Hsieh EW, Hernandez JD. Current Opinion in Immunology. 2021;72:298-308. doi:10.1016/j.coi.2021.07.010.

B cells

Agammaglobulinemia and B cell dysfunction
This case aims to highlight the essential role of B cells in adaptive immunity and antibody production.

Noah is a 2-year-old male who presents to the pediatrician with a history of recurrent bacterial infections since infancy.

By 6 months of age, he had already experienced multiple episodes of otitis media, with approximately 8 documented episodes per year. They required antibiotic treatment due to prolonged fevers and lack of improvement with watchful waiting. He was admitted for pneumonia at the age of 16 months after not responding to oral antimicrobials in the community and increased work of breathing, requiring intravenous antibiotics due to severe respiratory distress. During this hospitalization, blood cultures confirmed Haemophilus influenzae bacteremia.

You ask about family history and learn that Noah's parents are healthy, and that Noah has two older sisters who are healthy as well. Mom's brother passed away many years ago due to a severe infection at a young age, but she is unsure of the details.

Physical Examination: Noah appears well but pale, there are no palpable cervical lymph nodes in the inguinal area. There are no other significant findings on general physical examination.
You review chest X-rays from past infections and happy to see a small thymus with no significant abnormalities in lung fields.

Other lab investigations showed a WBC of 3.2 x 10^9/L and neutrophils of 0.8 x 10^9/L, with no signs of anemia and normal platelet counts. Flow cytometry revealed CD19+ B cells at 0.2% with no measurable B cell number, alongside normal CD3+ and NK counts. IgG levels were 1.5 g/L, while IgA and IgM were absent at 0.1 g/L each

Genetic testing confirmed X-linked agammaglobulinemia (XLA) due to a pathogenic variant in the BTK gene.

Questions:

1. What role do B cells play in immune defence and antibody production?
2. Describe the maturation stages of B cells.
3. How do defects in B cell function lead to immunodeficiency disorders?
4. What is the role of the spleen and lymph nodes in B cell immunity?
5. How are antibodies produced?
6. What is the role of each antibody?
7. What are the diagnostic criteria and management strategies for B cell-related immunodeficiencies?

Resources:

1. Janeway's Immunobiology
2. Basic Immunology, Abul K Abbas
3. https://www.youtube.com/watch?v=jPOGkuuvEu0 summary of the chapter on B cell activation and antibody production from Abbas.
4. https://www.youtube.com/watch?v=ZloOT8-AzQ8
5. https://www.youtube.com/watch?v=V6F5iBSHUBI
6. Fekrvand S et al. B- and T-Cell Subset Abnormalities in Monogenic Common Variable Immunodeficiency. Front Immunol. 2022 doi: 10.3389/fimmu.2022.912826.

Immunological Memory, B-cell Activation by T Cells

This case reviews the interaction between B-cell and T-cell in order for the adaptive immune system to function.

Abby is an 11-year-old female of European descent who presents to the Immunology Clinic with a history of recurrent pneumonia since early childhood. Each pneumonia would result in prolonged fever and cough despite appropriate courses of antibiotics.

Other history includes recurrent croup episodes in infancy, recurrent otitis media up to 10 times a year, and an episode of septic arthritis at the age of 8

Her recurrent pneumonias were attributed to poorly controlled asthma and IgA deficiency diagnosed in the community.

Her physical exam was unremarkable; lymph nodes were present and well palpated, no dysmorphic features and no skin or hair abnormalities.

Initial investigations at the age of 10 revealed mild leukopenia, and neutropenia of 0.9. Further evaluation showed borderline normal IgG, elevated IgM and undetectable IgA. Further work up showed non-reactive vaccine titers despite being vaccinated appropriately.

Genetic testing identified a homozygous variant in the CD40 gene, and flow cytometry showed a lack of expression of CD40 on B cells, confirming the diagnosis of hyper IgM syndrome.

1. This patient did not have any response to Tetanus and Diphtheria despite being vaccinated multiple times. Explain the role of immunological memory in adaptive immunity and how it is achieved.
2. Explain the process of immunoglobulin class switch recombination and B cell maturation accordingly
3. How does CD40 signaling contribute to immunological memory
4. What are the different factors that influence the choice of antibody isotype?

Resources:

1. Janeway's Immunobiology

2. Basic Immunology, Abul K Abbas
3. https://www.youtube.com/watch?v=uVr3LFoAJoo&t=474s
4. https://www.youtube.com/watch?v=V6F5iBSHUBI&t=179s
5. https://www.youtube.com/watch?v=CnjfthmW1ZM&t=54s
5. Yazdani R et al. The hyper IgM syndromes: Epidemiology, pathogenesis, clinical manifestations, diagnosis and management. Clin Immunol. 2019 Jan;198:19-30. doi: 10.1016/j.clim.2018.11.007.

Repertoire and Diversity

This case emphasizes the role of secondary diversification processes in the immune system

Emily Johnson, a 7-year-old female, presented with progressive difficulty in coordination and recurrent respiratory infections. She is currently followed by neurology and immunology for ataxia-telangiectasia (AT). Emily was diagnosed at the age of 4 years when genetic testing confirmed a known pathogenic variant in the ATM gene, establishing the diagnosis of AT. Her parents report that Emily began experiencing balance problems at the age of 3, which have worsened over time. Despite initially starting to walk on time, Emily has had many falls due to her unsteady gait and lack of coordination. She recently developed swallowing difficulties and is currently being considered for a G-tube to prevent aspirations and choking.

She has a history of recurrent respiratory infections, with approximately 10 episodes of ear infections and pneumonias requiring hospital admissions for intravenous antibiotics before starting IVIG replacement therapy. Her family faces additional challenges as Emily's mother struggles with breast cancer, adding emotional and logistical strain to their lives. There is no other family history of immunodeficiency or neurodegenerative diseases.

On physical examination, Emily exhibits a wide-based and unsteady gait. Telangiectasias are observed on her conjunctiva, face, and ears, and mild hepatosplenomegaly is noted. Laboratory findings reveal a normal white blood cell count with no anemia or thrombocytopenia. Her trough IgG level is 8, and she has low IgA with elevated IgM. Lymphocyte subsets show low-normal T cells and low B cells. Flow cytometry demonstrates reduced class-switched memory B cells.

Prior to starting IVIG, she was noted to have a poor response to Tetanus, Diphtheria, and polysaccharide vaccines, with low levels of specific antibodies post-immunization despite being vaccinated appropriately.

Emily's immunologist has advised her to avoid unnecessary exposure to X-rays due to concerns about the increased sensitivity of AT patients to ionizing radiation, which can further compromise their DNA repair processes.

Questions

1. Explain the process of V(D)J recombination and its significance in generating antibody diversity.
2. What is the significance of the ATM protein in DNA repair, and how does its dysfunction lead to immunodeficiency and other AT manifestations?
3. What is the role of germinal centers in the secondary diversification of the antibody repertoire?

4. How can the impaired antibody responses in A-T patients be managed clinically?
5. Emily's mother was just diagnosed with breast cancer. How is this related to the case?

Resources:

1. Janeway's Immunobiology
2. Basic Immunology, Abul K Abbas
3. VDJ recombination: https://www.youtube.com/watch?v=Bg5F1imauSo

Maturation of the T Lymphocyte cell.

Baby Timmy, a product of non-consanguineous parents, was delivered at 37 weeks gestation via elective c-section due to maternal gestational diabetes and hypertension. His mother was treated with labetalol and low dose aspirin since week 34 of gestation. The delivery was uncomplicated but Timmy experienced hypoglycemia after birth which resolved within days, as well as a rise in bilirubin and jaundice which resolved in a week.

Timmy has 2 healthy siblings. There is no history of immunodeficiency or severe infections in family members. His mother has learning disabilities and is struggling with mental health issues.
Newborn screening for severe combined immunodeficiency revealed low T cell receptor excision circle (TREC) levels in the patient's blood sample (53, cut-off: 75). This result triggered an immunology consult.

On examination, Timmy appeared alert with proper muscle tone and was positive for moro, stepping, and grasp reflex. He had a prominent nose and ear lobe folding. No heart murmurs were noted on auscultation. The rest of the physical assessment was normal.

The parents reported he was feeding well with a combination of breastmilk and formula. Other lab investigations showed a WBC of 8.23 with normal neutrophils and lymphocytes.

Lymphocyte count was within normal limits but immunophenotyping revealed low CD3+, CD4+, and CD8+ cells with increased CD19+ B cells and CD16+CD56+ NK cells. T cell function appeared normal with a PHA response within the normal range.

He continued to feed well and gain weight until he was 5 weeks old when he was admitted to the hospital with status epilepticus secondary to profound hypocalcaemia. One day before admission his FISH analysis was reported confirming a deletion in 22q11.2. He gradually improved in the hospital with calcium supplementation and anti-seizure medications.
At 6 months, he remained well overall. He did not experience any viral, bacterial or opportunistic infections. At 12 months, he showed appropriate responses to his killed vaccines and had further improvement in his lymphocyte count. He then received live viral vaccines. Two weeks after receiving the MMR vaccine, he presented to the ED with diffused petechiae, and his mom noted he had prolonged bleeding from his gums after brushing his teeth. CBC showed thrombocytopenia of 10 x 10^9/L with

normal hemoglobin and WBC. He was subsequently diagnosed with ITP, which resolved over the course of 4 months.

Questions:

1. What is the role of the thymus in immune system development?
2. How can thymic dysfunction lead to autoimmune disorders?
3. What are the mechanisms underlying T cell dysregulation in autoimmune diseases?
4. Describe positive and negative selection.
5. How do T cells contribute to the pathogenesis of autoimmune disorders?
6. What are some potential treatment options for thymic-related autoimmune disorders? (Describe how these meds work)

Resources:

1. Janeway's Immunobiology
2. Basic Immunology, Abul K Abbas
3. https://www.youtube.com/watch?v=geNZIRVVzUE
4. Mustillo PJ, et al. Clinical Practice Guidelines for the Immunological Management of Chromosome 22q11.2 Deletion Syndrome and Other Defects in Thymic Development. J Clin Immunol. 2023. doi: 10.1007/s10875-022-01418-y

Life cannot be sustained without T cells: Jacob, a 2-week-old with a Positive Newborn Screen

Jacob is a 2-week-old male infant who was identified by newborn screening to have low T-cell receptor excision circles (TREC = 5) and an abnormal purine profile, raising suspicion for adenosine deaminase deficiency severe combined immunodeficiency (ADA-SCID).

He is the first child of healthy Mennonite parents. He was born at 41 weeks by normal vaginal delivery after an uncomplicated pregnancy and perinatal course. He has been feeding well, gaining weight and was well-appearing at his first outpatient immunology visit.

Initial Investigations:
Newborn Screen:

- *TREC count*: 5 (low)
- *Deoxyadenosine*: 13.6 (high)
- Presumptive diagnosis: ADA-SCID

CBC: Leukopenia (1.4 x10⁹/L), lymphopenia (0.2 x10⁹/L), neutropenia (0.8 x10⁹/L) with normal Hb and platelets
Flow Cytometry: CD3, CD4, CD8: undetectable, CD19: <10 cells/µL and NK cells - 50 cells/µL

On his first visit the family was counseled on diagnosis of ADA-SCID, home isolation, ready-to-feed formula, and urgent care for infections. They were advised to avoid live viral vaccines and he was planned to initiate IVIG replacement therapy, prophylactic TMP-SMX, as well as to begin Enzyme Replacement Therapy with Revcovi.
Breastfeeding was discontinued due to maternal CMV seropositivity.

Despite precautions, Jacob developed a new cough and nasal congestion 4 days later. No fever or change in feeding/stooling, prompting ED assessment. He was afebrile, but had subcostal indrawing, RR 70s, O_2 sat 90–94%. He underwent a full septic workup, which came back negative. In-Hospital Assessment showed mild bronchiolitis-like changes on a chest X-ray.

Questions:

1. What is the mechanism of ADA-SCID, and why are all lymphocyte lineages affected?
2. What is the rationale for stopping breastfeeding even when breastmilk CMV PCR is negative?
3. What infectious organisms are you most concerned about in a neonate with SCID?
4. Why is it important to delay live vaccines in these infants?
5. What does the ADA gene normally do? What happens when ADA is deficient? How does ADA deficiency specifically impair T cell development and survival?
6. What is PAP, and why is it relevant in ADA-SCID?
7. How does enzyme replacement therapy (ERT) help?
8. What roles do CD4+ T cells and CD8+ T cells each play? How do T cells support B cell class switching and antibody production?
9. What should be the next recommendation in this child's care?

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1. Adenosine deaminase deficiency. National Library of Medicine (MedlinePlus)
2. Adenosine Deaminase (ADA)-Deficient Severe Combined Immune Deficiency (SCID): Molecular Pathogenesis and Clinical Manifestations. Bradford KL, Moretti FA, Carbonaro-Sarracino DA, Gaspar HB, Kohn DB. Journal of Clinical Immunology. 2017;37(7):626-637. doi:10.1007/s10875-017-0433-3.
3. Adenosine Deaminase Deficiency: A Review. Flinn AM, Gennery AR. Orphanet Journal of Rare Diseases. 2018;13(1):65. doi:10.1186/s13023-018-0807-5.
4. Consensus Approach for the Management of Severe Combined Immune Deficiency Caused by Adenosine Deaminase Deficiency. Kohn DB, Hershfield MS, Puck JM, et al. The Journal of Allergy and Clinical Immunology. 2019;143(3):852-863. doi:10.1016/j.jaci.2018.08.024.

Spleen - role and function

This case reviews the role of the spleen and its function to the immune system.

Carter is a 10-year-old African Canadian male who presents with recurrent infections and persistent fatigue. His medical history includes recurrent sinopulmonary bacterial infections, notably two episodes of pneumonia as well as one episode of meningitis within the past year.

His mother also reports persistent fatigue, pallor, and occasional pain crises, particularly in the abdomen and limbs.

The patient was diagnosed with sickle cell disease (HbSS) at birth through newborn screening and has a history of multiple hospitalizations due to vaso-occlusive crises. He has received all routine childhood vaccinations.

There is no family history of autoimmune diseases, although the father has sickle cell trait (HbAS).

The patient lives with his parents and two healthy siblings. He attends school regularly but has missed several days due to illness.

On physical examination, his temperature is 38.3°C, heart rate is 110 bpm, respiratory rate is 22 breaths per minute, and blood pressure is 100/60 mmHg. Pallor is noted on the conjunctiva and skin, with mild scleral icterus. There is no palpable splenomegaly.

Laboratory findings reveal a hemoglobin level of 75 mg/dL, a white blood cell count of 25 x 10^9/L, a platelet count of 450 x 10^9/L, and a reticulocyte count of 10%.
A peripheral smear shows the presence of sickle cells and Howell-Jolly bodies.
A chest X-ray indicates consolidation in the right lower lobe, consistent with pneumonia.

Questions:

1. What it the diagnosis of this patient from an immune standpoint and why?
2. How does the spleen contribute to the body's defense against bacterial infections?
3. Which types of immune cells are stored in the spleen?
4. Which specific pathogens are patients with functional asplenia particularly susceptible to?
5. What are Howell-Jolly bodies, and what do they indicate about splenic function?

Resources:

1. Janeway's Immunobiology
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Tolerance

This case highlights the importance of immune tolerance, particularly in the context of genetic mutations affecting central immune tolerance

Timmy TolOff is a three-year-old boy who was admitted with pain and swelling in both ankles and wrists, accompanied by a rash and fever lasting about six weeks.

He was referred for further evaluation of increased acute phase reactants, splenomegaly, and abdominal distension. On physical examination, Timmy had fever of 38.7°C and significant swelling, particularly in the right knee. He also had nail dystrophy and onychomycosis in the right thumb.

Timmy is the second child of consanguineous parents, who are third-degree cousin. The other two children in the family are healthy and have no reported health problems.

A preliminary diagnosis of systemic juvenile idiopathic arthritis (JIA) was considered due to the combination of fever, splenomegaly, rash, and arthritis lasting more than 10 days.
NSAIDs were started, leading to improvement in arthritis, fever, and rash after two weeks. However, Timmy developed candidiasis on the oral mucous membranes and tongue. Later that year, Timmy experienced cough and fatigue, resulting in hospitalization for pneumonia. During this hospital admission, he was also assessed for hypoparathyroidism with low calcium, high phosphorus, low PTH, and high 25OHD vitamin levels.

Laboratory findings were as follows:
CBC- normal WBC, mild neutropenia, normocytic anemia and normal platelets
CRP: 13.4 mg/dl (normal: 0–0.5)
Ferritin: 1435 ng/ml (normal: 7–140)
ESR: 26 mm/h (normal: <15 mm/h)
Positive ANA: 1/160 cytoplasmic
Negative Anti-DsDNA
Negative antistreptolysin O titer (ASO), rheumatoid factor (RF), and antineutrophil cytoplasmic antibody tests
No signs of hemophagocytosis, atypical cells, or blasts in his bone marrow aspiration
Lymphocyte subset analysis and T/B cell proliferation tests were normal
Genetic examination revealed a homozygous mutation in the AIRE gene. The parents were heterozygous for the same mutation.

Questions:

1. What are the primary mechanisms by which central immune tolerance is maintained in the thymus?
2. How does AIRE contribute to central immune tolerance?
3. What is the role of negative and positive selection in the thymus and how it prevents autoimmunity?
4. What else can be done for Timmy? Would he benefit from a stem cell transplant?

Resources:

1. Janeway's Immunobiology
2. Basic Immunology, Abul K Abbas
3. Pellegrino M et al. A Novel Homozygous Mutation of the AIRE Gene in an APECED Patient From Pakistan: Case Report and Review of the Literature. Front Immunol. 2018 doi: 10.3389/fimmu.2018.01835
4. https://www.youtube.com/watch?v=bWuupcEAmr8&t=793s (Immunologic Tolerance)
5. https://www.youtube.com/watch?v=YSwZ57HqyGg (Negative selection and AIRE)

McMaster University
Department of Pediatrics
3A-Health Sciences Centre
1280 Main Street West, Hamilton ON L8S 4K1
Tel: 905.521.2100
McMaster Children's Hospital
HAMILTON HEALTH SCIENCES

Navigating peripheral tolerance

This case highlights the critical role of regulatory T cells in maintaining immune tolerance and how FOXP3 mutations lead to severe autoimmune manifestations in IPEX syndrome

Quang, a 3-month-old male infant, presents with chronic diarrhea, poor weight gain, and a widespread eczematous rash. His parents report that he has had persistent loose stools since birth, requiring multiple formula changes without improvement. Additionally, he was recently diagnosed with type 1 diabetes mellitus after presenting with hyperglycemia and weight loss. His newborn screen was flagged for hypothyroidism, for which he has been treated since birth. His older maternal cousin reportedly passed away in infancy from an unknown autoimmune disorder in Vietnam a few years ago.

On physical examination, the infant appears irritable and cachectic with significant muscle wasting. His birth weight is below the 5th percentile. His skin shows generalized eczematous lesions with areas of excoriation and lichenification, particularly over the cheeks, flexural surfaces, and trunk. There is mild inguinal lymphadenopathy and hepatosplenomegaly. His abdomen is mildly distended but soft, with active bowel sounds. Cardiopulmonary examination is unremarkable.

The initial workup included a complete blood count which shows anemia and eosinophilia, along with mild thrombocytopenia. Serum chemistry reveals hypoalbuminemia and metabolic acidosis. An autoimmune panel demonstrates the presence of antinuclear antibodies, anti-thyroid peroxidase and islet cell autoantibodies. Stool studies are negative for infectious causes but reveal an increased fecal calprotectin level.

Flow cytometry analysis revealed a reduced population of regulatory T cells. Genetic testing confirms a pathogenic mutation in the FOXP3 gene, establishing the diagnosis of IPEX syndrome.

Questions:

1. What is the role of regulatory T cells (Tregs) in maintaining immune tolerance? Compare and contrast central and peripheral immune tolerance
2. How does the FOXP3 mutation contributes to the clinical picture of this patient?
3. Why does he have eosinophilia?
4. What can be done for Quang? Would he benefit from a stem cell transplant?
5. What are the major diagnoses that should be considered in his case? (Name 4-5 other IPEX-like syndromes affecting immune regulation)

Resources:

1. Bacchetta R et al. From IPEX syndrome to FOXP3 mutation: a lesson on immune dysregulation. Ann N Y Acad Sci. 2018 Apr;1417(1):5-22. doi: 10.1111/nyas.13011. PMID: 26918796.
2. Park JH et al. Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome: A systematic review. Autoimmun Rev. 2020 Jun;19(6):102526. doi: 10.1016/j.autrev.2020.102526. PMID: 32234571.
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McMaster University
Department of Pediatrics
3A-Health Sciences Centre
1280 Main Street West, Hamilton ON L8S 4K1
Tel: 905.521.2100
McMaster Children's Hospital
HAMILTON HEALTH SCIENCES

Failure of immunoglobulin class switching

Mateo is a 3-year-old boy presenting to the hospital with restlessness, constant purulent rhinorrhea and fevers for the past week. His mom suspects this is another bout of his many chest infections. Cultures were drawn, antibiotics were started, and he was admitted to general pediatrics.

His past medical history was significant for multiple sinus infections and a prolonged prior hospitalization for Pneumocystis jirovecii in Mexico. Mateo was adopted by his current family and just immigrated to Canada. His biological family history was otherwise unremarkable. After 7 days of IV antibiotics and successful resolution of his infection.
This history warranted an immunology consult.

Work up so far revealed as follows:
Nose, throat and blood cultures were positive group A streptococcus. His white blood counts was not elevated despite his significant infection initially and his automated differential revealed neutrophils at 16% with absolute neutrophil count of 1.1 $\times 10^9$ cells/L.
Post discharge, antibody titres to streptolysin O were measured and were non-reactive.

Quantitative immunoglobulins demonstrated very low IgG, undetectable IgA, and elevated IgM.
His vaccine titres that were non-reactive to measles, mumps, rubella, tetanus, and diphtheria, with minimal hepatitis B surface antibodies despite having been vaccinated repeatedly.
T and B cell immunophenotyping demonstrated normal naïve and non-switched memory B cells, very low class switched and memory B cells as well as an essentially normal T cell panel.

Whole exome sequencing was done at this point which identified a pathogenic variant in the CD40LG gene. Functional assay revealed normal expression of the CD40 protein on CD19 cells. However, CD40L expression on stimulated patient T cells were lower than that of control (9.9% in the patient compared to 19.4% in the control).

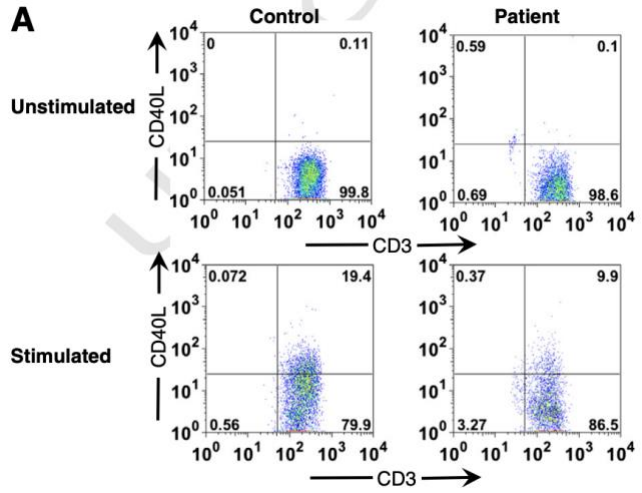

Figure 1 obtained from: Günaydin, N. C. et al. (2014). A novel disease-causing CD40L mutation reduces expression of CD40 ligand, but preserves CD40 binding capacity. Clinical Immunology, 153(2), 288–291. (Note: this is not the same case as presented case report with these results. Rather this figure is shown here as a sample result to interpret)

Resources:

1. What is the mechanism of the interaction between CD40 and CD40L?
2. In fluorescent activated cell sorting analysis, Mateo's B cells have surface IgM and IgD but no IgA and IgG. Can you explain why IgM to IgD isotype switching would not be affected?
3. Name 2 complications of CD40L deficiency that are possible for Mateo in the future if he does not undergo HSCT.
4. How would the functional assay for this patient look different from CD40 deficiency? Is there any clinical difference between CD40 and CD40L deficiency and why?

References:

1. Günaydin, N. C., Chou, J., Karaca, N. E., Aksu, G., Massaad, M. J., Azarsiz, E., … Kütükçüler, N. (2014). A novel disease-causing CD40L mutation reduces expression of CD40 ligand, but preserves CD40 binding capacity. Clinical Immunology, 153(2), 288–291.
2. Clinical, Genetic and Immunological Characteristics of 40 Chinese Patients With CD40 Ligand Deficiency. Du X, Tang W, Chen X, et al. Scandinavian Journal of Immunology. 2019;90(4):e12798. doi:10.1111/sji.12798
3. Human CD40 Ligand Deficiency Dysregulates the Macrophage Transcriptome Causing Functional Defects That Are Improved by Exogenous IFN-γ. Cabral-Marques O, Ramos RN, Schimke LF, et al. The Journal of Allergy and Clinical Immunology. 2017;139(3):900-912.e7. doi:10.1016/j.jaci.2016.07.018.

T Cell maturation in the thymus

This case highlights the process of thymus formation and T cell selection and maturation from the thymus.

Isla is a 2-week-old who initially presented to the immunology clinic as an urgent referral for positive newborn screen for SCID. Her recent history is significant for a brief NICU admission for hypoglycemia and self-resolving hyperbilirubinemia. Her T cell receptor excision circles (TREC) was 51 (lower limit: 75). At that point, this was repeated and found to be 120. Her family history was unremarkable for immunodeficiencies or immune dysregulation. Her parents are non-consanguineous. On further history, there was maternal uncle with a history of gastrointestinal issues requiring surgery, short stature qualifying for hormone therapy and also failure to thrive.

On initial blood work, her CBC was unremarkable. Lymphocyte subsets showed the following: CD3+CD4+ were low, CD3+CD8+ were low, CD19+ and CD16+CD56+ were normal. Analysis of the naïve/memory T cells (CD45RA/RO) was normal as well as the TCR repertoire.

Further testing was available from the newborn screen including the TBX1 assay (assay used to detect deletions or mutations in the TBX1 gene which are associated with DiGeorge syndrome), which did not detect any deletions. FISH for 22q11.2 was also normal.

A primary immunodeficiency gene panel was sent. Results identified a pathogenic variant in FOXN1 gene. Over time, she continued to have evidence of T-cell lymphopenia preferentially affecting CD3+ CD8+, but has improved over time. Her B and NK cells remained normal, and her functional assessment continued to be excellent for both the humoral and cellular arms. She was able to get her live viral vaccines at 18 months.

Questions:

1. What is the role of FOXN1 in thymic maturation?
2. What are TRECs and what do low levels signal?
3. How are DiGeorge syndrome and FOXN1 related (e.g. the initial work up for this patient)?
4. Why were the naïve/memory T cells normal in FOXN1 deficiency?
5. What are some physical exam findings that can be seen in FOXN1 deficiency (our case was negative for these)?

Resources:

1. Janeway's Immunobiology
2. Basic Immunology, Abul K Abbas
3. Bosticardo M et al. Heterozygous FOXN1 Variants Cause Low TRECs and Severe T Cell Lymphopenia, Revealing a Crucial Role of FOXN1 in Supporting Early Thymopoiesis. Am J Hum Genet. 2019 Sep 5;105(3):549-561. Link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6731368/
4. Very basic lymphoid organs review: https://www.khanacademy.org/science/how-does-the-human-body-work-class-12/x7babbc170453fdb8:human-health-and-disease/x7babbc170453fdb8:types-of-immunity-and-the-immune-system/a/lymphoid-organs-review

JAK STAT pathway

A 54-year-old woman presents to her family physician with recurrent oral thrush and nail infections. She has had these infections intermittently since her teenage years. Despite treatment with antifungals, her symptoms keep returning.
She also carries a history of type 1 diabetes mellitus (T1DM) (diagnosed at 14) and hypothyroidism (diagnosed at 20). Over the years, she developed esophageal candidiasis with stricturing, recurrent vaginal yeast infections, and one episode of severe paronychia requiring IV antifungals.
She insists that her blood sugars are generally well controlled, yet clinicians often attributed her infections to her diabetes.
Her mother had Hashimoto's thyroiditis and chronic fungal nail infections, dying at age 74 of unclear cause. At age 48, she was referred to Allergy & Clinical Immunology after developing urticaria to fluconazole. The allergist noted her autoimmunity plus chronic mucocutaneous candidiasis (CMCC) and initiated further workup.

Laboratory results showed as follows:

- Low-normal lymphocyte counts (CD3+ T cells reduced, particularly CD4+).
- Normal immunoglobulins.
- Protective vaccine titers to most antigens.
- Negative varicella IgG despite vaccination.

Genetic testing revealed a novel heterozygous missense mutation in STAT1 (c.842A>G, p.E281G). Functional assays showed persistent STAT1 hyperphosphorylation after cytokine stimulation, confirming a STAT1 gain-of-function (GOF) mutation.
The patient was started on ruxolitinib, a JAK inhibitor. After 5 months, her candidiasis and lymphopenia improved significantly.

Questions:

1. Review the JAK–STAT signaling pathway:
    1. How is STAT1 normally activated and inactivated?
    2. What happens when STAT1 is overactive?
2. Why does STAT1 GOF lead to both autoimmunity and fungal susceptibility?
3. How does impaired Th17 immunity connect to Candida infections?
4. How do JAK inhibitors help in STAT1 GOF?
5. What are the risks of long-term JAK inhibitor use?
6. What are other IEIs that can present with endocrine autoimmunity and chronic infections?

References:

1. Ott N, Faletti L, Heeg M, Andreani V, Grimbacher B. JAKs and STATs from a Clinical Perspective: Loss-of-Function Mutations, Gain-of-Function Mutations, and Their Multidimensional Consequences. J Clin Immunol. 2023 Aug;43(6):1326-1359. doi: 10.1007/s10875-023-01483-x. Epub 2023 May 4. PMID: 37140667
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3. Asano T, Noma K, Mizoguchi Y, Karakawa S, Okada S. Human STAT1 gain of function with chronic mucocutaneous candidiasis: A comprehensive review for strengthening the connection between bedside observations and laboratory research. Immunol Rev. 2024 Mar;322(1):81-97. doi: 10.1111/imr.13300. Epub 2023 Dec 12. PMID: 38084635.
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5. Tzeng HT, Chyuan IT, Lai JH. Targeting the JAK-STAT pathway in autoimmune diseases and cancers: A focus on molecular mechanisms and therapeutic potential. Biochem Pharmacol. 2021 Nov;193:114760. doi: 10.1016/j.bcp.2021.114760. Epub 2021 Sep 4. PMID: 34492272.

McMaster University
Department of Pediatrics
3A-Health Sciences Centre
1280 Main Street West, Hamilton ON L8S 4K1
Tel: 905.521.2100
McMaster Children's Hospital
HAMILTON HEALTH SCIENCES

Th17 pathway

The aim of this case is to review the Th17 pathway and neutrophil recruitment.

Stata is a 5 year-old girl who presents with recurrent infections since infancy.
Her parents report multiple episodes of skin abscesses which were attributed to her bad eczema.
She also has a history of recurrent pneumonia and one hospital admission for pneumonia complicated by pleural effusion and abscess. She was treated for asthma by her family doctor with ICS and bronchodilators as needed since infancy.
Additionally, she recently developed oral thrush. This was first thought to be due to the use of inhaled corticosteroids for her asthma, however this had persisted despite rinsing her mouth after every use and local anti-fungal medications.

Her mother tells you that she keeps getting sick despite appropriate antibiotic therapy. Last year she was admitted with osteomyelitis of the proximal tibia and associated Staph Aureus bacteremia treated with IV antibiotics.. Interestingly, this has started initially as a skin infection after a mosquito bite.

Her growth and development have been normal, with no cognitive deficits noted.
On review of systems, you learn she is waiting for an assessment with orthopedics for suspected scoliosis as see on x-rays and on physical exam.
There is no family history of immunodeficiency or atopy, she has a healthy older brother.

On physical exam, Stata appears well-nourished with normal vital signs. You note she has multiple healed scars from previous abscesses on the extremities. There are no dysmorphic features, but you suspect her nose might be prominent, unlike her parents. The rest of her exam is unremarkable.

<u>Investigations showed as follows:</u>
Normal CBC
Normal IgG, IgM, IgA, elevated serum IgE levels 17,000 IU/mL
Lymphocyte subsets did not show any T or B cells abnormalities
Chest CT scan reveal evidence of bronchiectasis with multiple dilated and thick-walled bronchi bilaterally. There are also areas of ground-glass opacities suggestive of chronic inflammation and recurrent pneumonias.
While you wait for genetic testing results, immunological workup reveals impaired T-helper 17 (Th17) cell differentiation and defective neutrophil chemotaxis.

Questions:

1. What is the differential diagnosis for this possible immunodeficiency? Base this on molecular mechanisms of Hyper IgE syndrome.
2. What is the diagnosis in this case based on all the clinical clues provided?
3. Why did she develop both candidiasis and bacterial infections?
4. Describe the different subsets of T-helper cells (Th1, Th2, Th17) and their roles in the immune response.
5. What is the function of the STAT3 protein in normal immune signaling pathways?

Resources:

1. Janeway's Immunobiology
2. Basic Immunology, Abul K Abbas
3. Differentiation of T cells into CD4 effector cells: https://www.youtube.com/watch?v=lAxakO-pzvc
4. Hyper IgE syndromes: clinical and molecular characteristics. Taha Al-Shaikhly et al, 2018, Immunology & Cell Biology. https://doi.org/10.1111/imcb.12209

Neutrophil migration/adhesion in LAD and T effector cell subsets

This case is designed to integrate foundational immunology concepts with clinical transplant immunopathology. It highlights how a primary defect in neutrophil adhesion and migration, as seen in transitions into a T cell–driven immune dysregulation following hematopoietic stem cell transplant.
To optimize learning, it is recommended to divide the discussion into two sections: Neutrophil Migration and Innate Immunity in LAD-I and T cell effector mechanisms in GVHD

Ramzy, a 6-year-old boy of Syrian descent, has a confirmed diagnosis of LAD-I due to a known mutation in ITGB2/CD18. He undergoes an allogeneic hematopoietic stem cell transplant from a mismatched unrelated donor. Pre-transplant, he had recurrent omphalitis and delayed separation of the umbilical cord, with persistent leukocytosis and high circulating neutrophil counts.
He receives myeloablative conditioning and post-transplant cyclophosphamide as GVHD prophylaxis. At day +30 post-transplant, he develops pruritic erythematous rash on palms and trunk, profuse watery diarrhea.

Before transplant, Ramzy's bloodwork showed marked leukocytosis with a white blood cell count of 55 × 10^9/L and absolute neutrophil count 45 × 10^9/L. Flow cytometry demonstrated less than 2% CD18 expression on neutrophils, confirming the functional adhesion defect. His hemoglobin was mildly reduced at 110 g/L, platelets were normal at 420 × 10^9/L, and C-reactive protein was elevated at 45 mg/L

Post-transplant labs: chimerism studies showed 95% donor engraftment. The WBC had normalized to 8.5 × 10^9/L, with an absolute neutrophil count of 4.2 × 10^9/L and lymphocytes of 3.0 × 10^9/L.
Liver enzymes were elevated: ALT 85 U/L and AST 70 U/L while bilirubin and albumin remained within normal limits. Stool studies were negative for infectious pathogens.

Questions:

- What is the molecular defect in LAD-I, and how does impaired CD18 (β2 integrin) expression disrupt neutrophil adhesion, migration, and microbial clearance?
- How do these defects explain Ramzy's pre-transplant findings (persistent leukocytosis, recurrent infections, delayed cord separation)?
- Which clinical and laboratory findings in this case suggest early acute GVHD, and what immunologic mechanisms underlie these manifestations?

- How do donor-derived T cells recognize and damage host tissues after transplant?
- What T effector cell subsets (Th1, Th17, cytotoxic T cells) are involved in GVHD, and what molecular signals (antigen presentation, cytokines, costimulation) drive their activation and differentiation?
- How do cytokines such as IFN-γ, TNF-α, and IL-17 amplify the inflammatory cascade and tissue injury in acute GVHD?
- How might the innate immune defect in LAD-I influence immune reconstitution and susceptibility to post-transplant complications like GVHD or infection?
- What immunomodulatory strategies (e.g., post-transplant cyclophosphamide, Treg expansion) specifically target T effector cell activation or proliferation to prevent GVHD?

Defect in immune response to a virus

This case highlights the importance of functional B and T cell interaction in response to viruses.

Casey is a 5-year-old boy who was brought to the ER by his parents due to a 2-week history of fevers, 4-day history of fatigue and sore throat.

Initially into his illness course, he only had the fever with no other symptoms and was managed with Acetaminophen as needed. After 1 week, his energy was lower, and he became weak. He also had developed a concerning petechial rash in the past day, which prompted parents to bring him to the ER. His exam in the ER was significant for a fever of 39.2C, significant axillary, cervical and supraclavicular non-tender lymphadenopathy and a non-exudative erythematous oropharynx. He was also noted to have hepatosplenomegaly.

His past medical history was significant for at least 5 to 6 recurrent ear infections and 3 episodes of pneumonia (2 of which were confirmed on chest x-ray) requiring prolonged antibiotic management. His birth history was otherwise unremarkable. He was born to non-consanguineous parents of Caucasian descent. His family history was significant for a maternal uncle that died at age 7 due to an unknown viral infection. Otherwise, there was no known history of immunodeficiencies or immune dysregulation.

Initial blood work had revealed anemia, thrombocytopenia, normal WBC, elevated AST and ALT, hyperbilirubinemia as well as markedly elevated ferritin and triglycerides. Given the enlarged lymph nodes and sore throat, EBV serologies were sent; EBV IgM antibodies against EBV viral capsid antigen (VCA) positive at more than 1:40. Anti-VCA IgG was negative. EBV DNA PCR was positive at 30,000 copies/mL. A chest x-ray revealed large mediastinum with lymphadenopathy and US of the abdomen revealed hepatomegaly with mild ascites. A CT abdomen also revealed enlarged lymph nodes in the retroperitoneum. At this point, immunology was consulted.

Given the concern for an EBV susceptibility syndrome with fulminant EBV infection and resulting HLH, he was started on ganciclovir and IVIG. Broad spectrum antibiotics were also initiated to prevent secondary infections. Lastly, he needed IV methylprednisolone to manage the hyperinflammatory response.

His initial immunology work up demonstrated low quantitative IgG but normal IgM and IgA. His T and B cell subsets demonstrated the following (all values are in $x10^9$ cells/L).
CD3 2.89 (high), CD4 0.54, CD8 2.32 (high), CD4/CD8 0.2 (low)
CD19 <0.01 (low), CD16/CD56 0.03 (low)

Casey eventually recovered with supportive care. His inborn errors of immunity panel demonstrated a pathogenic a hemizygous pathogenic variant in SH2D1A gene, confirming the diagnosis of X-linked lymphoproliferative (XLP) disorder. He was referred for evaluation of hematopoietic stem cell transplantation.

Questions:

1. What is the role of SAP (SLAM-associated protein) in T and B cell interactions, and how does its deficiency affect immune function?
2. What are 2-3 immune responses which would be altered due to loss of function of SAP?
3. Explain the impact of defected cytotoxic T lymphocyte and Natural Killer cell on the rest of the immune system, and how this impairment contributes to the clinical presentation of SAP-deficient patients
4. Casey has a 3-year-old brother who also has SAP deficiency. What can be done to prevent the progression of severe disease in this boy?
5. How does the inability to clear EBV translate to a lymphoproliferative disorder?
6. Would it be possible for a female with SH2D1A pathogenic variant to present with fulminant EBV? What are other possibilities in that case?
7. Describe other genetic syndromes that increase susceptibility to EBV infections

Resources:

1. Janeway's Immunobiology
2. Basic Immunology, Abul K Abbas
3. Meyer L, Hines M, Zhang K, et al. X-Linked Lymphoproliferative Disease. 2004 Feb 27 [Updated 2024 May 16]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. https://www.ncbi.nlm.nih.gov/books/NBK1406/

Asthma, eczema, Th2 inflammation

This case aims to deepen understanding of the immunological pathways of severe allergic diseases

Jack is a 15-year-old male, presenting with several concerning symptoms to your community Allergy clinic.

Jack experiences multiple episodes of recurrent wheezing in the past few months. He experiences symptoms during the day and night almost daily and has significant limitations in physical activities due to his symptoms. He has required one hospitalization due to asthma exacerbation in the past year after contracting human metapneumovirus.

He also tells you that his symptoms are exacerbated by the pollen season, exposure to dust when he is cleaning his room, and visits to his grandparents' house who have a cat. This is also accompanied by sneezing and nasal congestion.

His asthma symptoms started around age 2 years, initially with mild intermittent wheezing during viral infections. This progressively worsened, leading to frequent episodes requiring multiple different inhaled bronchodilators and corticosteroids. Last year, he was admitted to the PICU due to a severe asthma attack requiring high flow nasal cannula
His symptoms of eczema began in early childhood, characterized by intense pruritus, multiple erythematous patches, and lichenification in flexural areas. Despite regular use of emollients and topical corticosteroids, his eczema has been difficult to control, leading to intermittent courses of antibiotics during severe exacerbations prescribed by his family doctor.

There are no food allergies, but he does note that he has some mouth itch when he eats certain raw fruits and vegetables. His family history is remarkable for allergic rhinitis in both parents. He has a younger sister who has eczema. Skin prick tests confirmed sensitization to cat, grass, birch and house dust mite. Spirometry demonstrated reversible airflow obstruction suggestive of asthma.

Jack and his parents expressed their frustration about Jack's severe asthma and eczema. He feels embarrassed by his extensive skin rash and thinks his mom thinks he might be developing depression and anxiety since being discharged from the PICU.
Questions:

1. How does the skin contribute to the immune system's defense mechanisms?
2. What are the mechanisms underlying allergic inflammation in the skin and lungs? Which cells are participating? Name key players, cytokines and their function
3. What would be a treatment strategy in this case? Why? What are possible side effects? Compare and contrast the different options.

Resources:

4. Janeway's Immunobiology
5. Basic Immunology, Abul K Abbas
6. Before diving into Th2 inflammation, you might benefit from looking into differentiation of T cells into CD4 effector cells: https://www.youtube.com/watch?v=lAxakO-pzvc
7. Biologic Therapies for Severe Asthma. Guy G. Brusselle et al. 2022, N Engl J Med 2022;386:157-171. DOI: 10.1056/NEJMra2032506
8. https://www.youtube.com/watch?v=MLpX_XiTb9Q&t=1757s
9. Immunobiology of IgE antibodies: https://www.youtube.com/watch?v=miwrd6yNKiM

Allergy and Immunotherapy in a Teen with autoimmune disease

This case highlights the approach to allergic rhinitis with a nuanced understanding of immunotherapy, especially in patients with comorbidities requiring immunosuppression.

Sophia, a 16-year-old female, presents to the allergy clinic with allergic rhinitis. She reports symptoms of nasal congestion, runny nose, and itchy eyes, which she experiences year-round, although symptoms are worse in spring and fall. She has a "hypoallergenic" dog at home but does not think it contributes to her symptoms.

On examination, Sophia has pale, swollen nasal turbinates and clear nasal discharge. Mild tenderness is noted on sinus palpation. She has mild periorbital darkening and conjunctival injection with watery eyes. Skin examination reveals scattered patches of dry, eczematous areas on the antecubital fossae without active inflammation. Her lungs are clear to auscultation with no wheezing. Abdominal examination reveals mild tenderness in the lower quadrants without guarding or distension.
Her skin prick testing was positive for house dust mite, grass, tree mix, birch, dog, and cat.

Sophia states that she has tried multiple antihistamines and nasal sprays, which were not helpful. Recently, she has had trouble sleeping due to nasal congestion and feels that this has affected her school performance. She tells you she would like to try subcutaneous immunotherapy.

Questions:

1. Describe the immunologic process of sensitization in allergic rhinitis, including the roles of IgE, mast cells, Th2 cytokines, and allergen-specific T helper cells. Explain how repeated allergen exposure leads to an immune response and symptoms of allergic rhinitis.
2. Explain how allergen immunotherapy works to shift the immune response towards tolerance.
3. Consider how immunomodulatory therapies, such as allergen immunotherapy, may influence immune balance in patients with underlying inflammatory conditions, as in Sophia's case.
4. Explore the impact of anti-TNF therapy on immune pathways, particularly in patients with chronic inflammatory diseases like IBD. Would that interfere with SCIT?
5. Can TNF inhibition affect the efficacy and safety of allergen immunotherapy?
6. Sophia wants to know if she is at risk of developing asthma and if immunotherapy can prevent that from happening.
7. How might "hypoallergenic" pets still contribute to allergic symptoms?

Resources:

1. Dykewicz MS et al Rhinitis 2020: A practice parameter update. J Allergy Clin Immunol. 2020 Oct;146(4):721-767. doi: 10.1016/j.jaci.2020.07.007. Epub 2020 Jul 22. PMID: 32707227.
2. Matsui EC et al. Indoor Environmental Control Practices and Asthma Management. Pediatrics. 2016 Nov;138(5):e20162589. doi: 10.1542/peds.2016-2589. PMID: 27940791.
3. Eggleston PA et al. Environmental allergen avoidance: an overview. J Allergy Clin Immunol. 2001 Mar;107(3 Suppl):S403-5. doi: 10.1067/mai.2001.113673. PMID: 11242600.
4. Salinas GF et al. Anti-TNF treatment blocks the induction of T cell-dependent humoral responses. Ann Rheum Dis. 2013 Jun;72(6):1037-43. doi: 10.1136/annrheumdis-2011-201270. Epub 2012 Sep 11. Erratum in: Ann Rheum Dis. 2018 Jan;77(1):158. doi: 10.1136/annrheumdis-2011-201270corr1. PMID: 22968102.

5. Buhre JS et al. Anti-TNF therapy impairs both short- and long-term IgG responses after repeated vaccination. Allergy. 2024 Jul 25. doi: 10.1111/all.16241. Epub ahead of print. PMID: 39049686.
6. Burgess JA et al. Childhood allergic rhinitis predicts asthma incidence and persistence to middle age: a longitudinal study. J Allergy Clin Immunol. 2007 Oct;120(4):863-9. doi: 10.1016/j.jaci.2007.07.020. Epub 2007 Sep 7. PMID: 17825896.
7. Schmitt J et al. Allergy immunotherapy for allergic rhinitis effectively prevents asthma: Results from a large retrospective cohort study. J Allergy Clin Immunol. 2015 Dec;136(6):1511-1516. doi: 10.1016/j.jaci.2015.07.038. Epub 2015 Sep 12. PMID: 26371838.

Severe T cell deficiency

This case highlights the process of thymus formation and T cell selection and maturation from the thymus.

Isla is a 2-week-old who initially presented to the immunology clinic as an urgent referral for positive newborn screen for SCID. Her recent history is significant for a brief NICU admission for hypoglycemia and self-resolving hyperbilirubinemia. Her T cell receptor excision circles (TREC) was 51 (lower limit: 75). At that point, this was repeated and found to be 120. Her family history was unremarkable for immunodeficiencies or immune dysregulation. Her parents are non-consanguineous. On further history, there was maternal uncle with a history of gastrointestinal issues requiring surgery, short stature qualifying for hormone therapy and also failure to thrive.

On initial blood work, her CBC was unremarkable (no leukopenia). Lymphocyte subsets showed the following: CD3+CD4+ were low, CD3+CD8+ were low, CD19+ and CD16+CD56+ were normal. Analysis of the naïve/memory T cells (CD45RA/RO) was normal.

Further testing was available from the newborn screen including the TBX1 assay (assay used to detect deletions or mutations in the TBX1 gene which are associated with DiGeorge syndrome), which did not detect any deletions. FISH for 22q11.2 was also normal.

A primary immunodeficiency gene panel was sent. Results identified a pathogenic variant in FOXN1 gene. Over time, she continued to have evidence of T-cell lymphopenia preferentially affecting CD3+ CD8+, but has improved over time. Her B and NK cells remained normal, and her functional assessment continued to be excellent for both the humoral and cellular arms. She was able to get her live viral vaccines at 18 months.

Questions:

6. What is the role of FOXN1 in thymic maturation?
7. What are TRECs and what do low levels signal?
8. How are DiGeorge syndrome and FOXN1 related (e.g. the initial work up for this patient)?
9. Why were the naïve/memory T cells normal in FOXN1 deficiency?
10. What are some physical exam findings that can be seen in FOXN1 deficiency (our case was negative for these)?

Resources:

5. Janeway's Immunobiology
6. Basic Immunology, Abul K Abbas
7. Bosticardo M et al. Heterozygous FOXN1 Variants Cause Low TRECs and Severe T Cell Lymphopenia, Revealing a Crucial Role of FOXN1 in Supporting Early Thymopoiesis. Am J Hum Genet. 2019 Sep 5;105(3):549-561. Link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6731368/

Very basic lymphoid organs review: https://www.khanacademy.org/science/how-does-the-human-body-work-class-12/x7babbc170453fdb8:human-health-and-disease/x7babbc170453fdb8:types-of-immunity-and-the-immune-system/a/lymphoid-organs-review

Major histocompatibility complex

Mina, a 10-year-old girl is referred to a tertiary pediatric immunology clinic with a long-standing history of dyspnea and recurrent productive cough. She has suffered from chronic sinus infections and recurrent pneumonia since the age of four. Despite multiple courses of antibiotics and bronchodilator therapy, her symptoms have persisted.

She is the eighth child of first-degree consanguineous parents, with an unremarkable family history except for a brother who was diagnosed with bronchiectasis.

Physical examination findings:

- Purulent post-nasal discharge
- Decreased breath sounds and dullness over the right hemithorax
- Clubbing of fingers

Initial investigations:

- Chest X-ray and chest CT: Right middle lobe atelectasis and bronchiectasis
- Pulmonary function testing: Reversibility of 22% with bronchodilators
- Bronchoscopy: Purulent secretions; bronchoalveolar lavage grew Haemophilus influenzae
- Negative for cystic fibrosis, primary ciliary dyskinesia, gastroesophageal reflux, and foreign body aspiration

A 21-year-old man, Mina's older brother is also referred for evaluation due to a history of recurrent respiratory infections and established bronchiectasis.

The patient reports a lifelong history of productive cough and recurrent pneumonia beginning in childhood. Chronic sinusitis and nasal congestion. Progressive decline in lung function over the past few years. He was previously diagnosed with bronchiectasis at another medical center in the Middle East but had not undergone a detailed immunological workup.

His physical examination findings are remarkable for digital clubbing and bilateral coarse crackles on lung auscultation, nasal congestion with purulent post-nasal discharge. No hepatosplenomegaly or lymphadenopathy.

CBC showed normal Hb, WBC 6.4, Plt 291 with normal absolute lymphocyte and neutrophil counts. IgA, IgM, IgG and IgE were within the normal range, however, with no antibody responses to protein or

polysaccharide antigens. Flow cytometry showed normal numbers of CD3, CD4 positive cells, slightly low CD8 positive cells, and normal B and NK cells.

Given the family history, you decide to complete genetic testing. A genetic panel identified the same homozygous TAP1 pathogenic variant in both siblings, leading to a frameshift mutation and premature stop codon.

Questions:

1. Describe the structure and function of MHC class 1 and 2
2. How does a mutation in TAP1 result in low HLA class I expression?
3. Why might a diagnosis of MHC class I deficiency be delayed in some patients?
4. What additional immune evaluations (not stated in the case) should be considered for patients with MHC class I deficiency?
5. Describe management for both patients
6. How does the management of bronchiectasis in these patient differ from standard management in non-immunodeficient individuals?

Resources:

1. Zimmer J, Andrès E, Donato L, Hanau D, Hentges F, de la Salle H. Clinical and immunological aspects of HLA class I deficiency. QJM. 2005 Oct;98(10):719-27. doi: 10.1093/qjmed/hci112. Epub 2005 Aug 8. PMID: 16087697.
2. Hanna S, Etzioni A. MHC class I and II deficiencies. J Allergy Clin Immunol. 2014 Aug;134(2):269-75. doi: 10.1016/j.jaci.2014.06.001. Epub 2014 Jul 4. PMID: 25001848.
3. Tsilifis C, Moreira D, Marques L, Neves E, Slatter MA, Gennery AR. Stem cell transplantation as treatment for major histocompatibility class I deficiency. Clin Immunol. 2021 Aug;229:108801. doi: 10.1016/j.clim.2021.108801. Epub 2021 Jul 16. PMID: 34280577.
4. 08 Immunology: MHC Molecules and Antigens (Domen)

Immunomodulators and Biologics

Jia, a 24-year-old female presents to the immunology clinic with worsening fatigue, intermittent fevers, and joint pain. She has a history of juvenile idiopathic arthritis (JIA) diagnosed at age 7 and was initially treated with methotrexate. Over time, she has been treated with various biologics, including adalimumab, tocilizumab, and more recently, abatacept.
Two years ago, she was assessed for recurrent sinopulmonary infections and an overwhelming disseminated mucocutaneous HSV infection, leading to a hospital admission due to dehydration. She was diagnosed with hypogammaglobulinemia and absent vaccine titers despite being vaccinated as per the Ontario vaccination schedule, for which she started intravenous immunoglobulin therapy.

On examination, she appears pale, with mild synovitis in both wrists and knees. She has mild splenomegaly and mild cervical lymphadenopathy.

Laboratory findings show mild anemia, lymphopenia, and elevated inflammatory markers. Immunoglobulin levels are low despite IVIG therapy.

Questions:

1. Compare and contrast the roles of CD4+ T cells and CD8+ T cells in the immune response in the context of this patient. What are the different cytokines involved in each pathway?
2. How methotrexate affects the immune function? What is the mechanism?
3. What are the mechanisms of action of adalimumab, tocilizumab, and abatacept?
4. How does blocking TNF-α, IL-6, or CTLA-4 signaling alter immune responses?
5. Jia's genetic testing revealed a pathogenic variant in TNFRSF13B (encoding TACI). What is the clinical significance?
6. How does a mutation in TNFRSF13B contribute to immune dysregulation and recurrent infections?
7. What are the potential reasons for Jia's continued low immunoglobulin levels despite receiving IVIG therapy?

Mast Cells physiology

Eden, a 28-year-old woman, came to the Allergy clinic complaining of recurrent episodes of severe itching, hives, flushing and abdominal pain which had been bothering her for the past two years. She believes that these episodes often triggered by stress, certain foods, or environmental changes.

Over the past year, the frequency and intensity of her symptoms had increased, with episodes now including dizziness, abdominal cramping, and occasional difficulty breathing. While these symptoms were usually alleviated by antihistamines, the relief was only temporary, and Eden had noticed that her symptoms were becoming harder to manage.
Eden had no known history of allergies, asthma, or any other chronic medical conditions. She denied any known food, venom or drug allergies, and her family history was unremarkable for similar symptoms or diagnoses.

On examination, Eden appeared well-nourished with normal vital signs.
Pictures she had taken previously of her skin showed multiple urticarial lesions on her arms and torso, some of which were in the process of resolving.
The rest of her physical exam was normal, and there was no sign of lymphadenopathy or hepatosplenomegaly.
Initial lab work revealed a normal complete blood count and immunoglobulins level, including IgE. Her tryptase levels were elevated at 26 ng/mL, (upper normal range of less than 11 ng/mL)

With these findings you suspect a possible disorder of mast cell activation. To rule out other conditions, you would like to order further investigations.

Questions

- What is the role of mast cells?
- How their inappropriate activation leads to symptoms such as urticaria, anaphylaxis, and gastrointestinal disturbances (review mediators released from mast cells)
- How are mast cells being activated? Review the various stimuli (e.g., IgE, complement, direct triggers).
- Her tryptase levels are elevated, but her bone marrow biopsy is normal. What does this suggest about her diagnosis?
- Explore management strategies

Resources

1. Jackson CW et al. Mastocytosis and Mast Cell Activation Disorders: Clearing the Air. Int J Mol Sci. 2021 Oct 19;22(20):11270. doi: 10.3390/ijms222011270. PMID: 34681933
2. Valent P et al. Diagnosis and classification of mast cell proliferative disorders: delineation from immunologic diseases and non-mast cell hematopoietic neoplasms. J Allergy Clin Immunol. 2004 Jul;114(1):3-11; quiz 12. doi: 10.1016/j.jaci.2004.02.045. PMID: 15241337.
3. Akin C. How to evaluate the patient with a suspected mast cell disorder and how/when to manage symptoms. Hematology Am Soc Hematol Educ Program. 2022 Dec 9;2022(1):55-63. PMID: 36485101

The Apoptosis Breakdown – Autoimmune Lymphoproliferative Syndrome (ALPS)

This case highlights the pathophysiology of ALPS, particularly the role of defective apoptosis in immune dysregulation.

Alex, a 7-year-old boy, is referred to the pediatric hematology clinic for evaluation of chronic lymphadenopathy and splenomegaly. His parents report that his lymph nodes have been persistently enlarged since infancy, and he has experienced recurrent episodes of pallor, fatigue, and easy bruising. His past medical history is significant for autoimmune hemolytic anemia diagnosed at the age of 5 and episodes of thrombocytopenia. His family history is notable for an older cousin who also had chronic lymphadenopathy and anemia of unclear etiology.
On physical examination, the child appears well but has generalized, non-tender cervical, axillary, and inguinal lymphadenopathy. His spleen is palpable 4 cm below the left costal margin, and hepatomegaly is noted. There are no signs of acute infection.

Laboratory Findings:

- Complete blood count: Hemoglobin 8.5 g/dL, platelets 90 × 10^9/L, white blood cell count 8.0 × 10^9/L (normal)
- Reticulocyte count: 5.5% (elevated)

- Direct Coombs test: Positive
- Flow cytometry: Elevated double-negative T cells (CD3+TCRαβ+CD4−CD8−)
- Elevated IL-10- 15 pg/mL (normal <5 pg/mL) and vitamin B12 levels 1200 pmol/L (normal range ~150-650 pmol/L)
- Genetic testing: Pathogenic variant detected in the FAS gene (c.786+1G>A)

Questions:

1. What is the underlying pathophysiology of ALPS, and how does it contribute to the patient's symptoms?
2. What is the role of apoptosis in immune homeostasis, and how does its failure contribute to autoimmunity?
3. How do Fas-FasL interactions regulate lymphocyte survival?
4. What mechanisms prevent self-reactive lymphocytes from causing autoimmunity under normal conditions?
5. How does the presence of double-negative T cells (CD3+TCRαβ+CD4−CD8−) assist in the diagnosis of ALPS? What are the diagnostic criteria for ALPS, and how does this patient fulfill them?
6. What role do genetic mutations, particularly in the FAS pathway, play in ALPS pathogenesis?
7. What are the main treatment options for ALPS, and why therapies such as sirolimus be considered?
8. What is the DDx of ALPS from a genetics standpoint?
9. What are the long-term complications of ALPS, and how should this patient be monitored over time?

Resources:

1. Price S et al. Natural history of autoimmune lymphoproliferative syndrome associated with FAS gene mutations. Blood. 2014 Mar 27;123(13):1989-99. Epub 2014 Jan 7. PMID: 24398331
2. Paskiewicz A et al. Autoimmune lymphoproliferative syndrome: A disorder of immune dysregulation. Autoimmun Rev. 2023 Nov;22(11):103442. doi: 10.1016/j.autrev.2023.103442. Epub 2023 Sep 6. PMID: 37683818.
3. Matson DR, Yang DT. Autoimmune Lymphoproliferative Syndrome: An Overview. Arch Pathol Lab Med. 2020 Feb;144(2):245-251. Epub 2019 Apr 8. PMID: 30958694; PMCID: PMC10415410.
4. Li P et al. Updated Understanding of Autoimmune Lymphoproliferative Syndrome (ALPS). Clin Rev Allergy Immunol. 2016 Feb;50(1):55-63. PMID: 25663566.

T-cell-Dependent B-cell Activation

An 18-Year-Old with Recurrent Infections and Autoimmunity due to inducible T-cell co-stimulator (ICOS) deficiency

Aidan an 18-year-old male university student, presents to the immunology clinic for re-evaluation of recurrent infections and autoimmune complications. He has a history of frequent bacterial sinusitis, multiple episodes of pneumonia requiring hospitalization, and chronic diarrhea with weight loss over the

past five years. At age 14, he was diagnosed with common variable immunodeficiency due to persistent hypogammaglobulinemia and poor vaccine responses and started IVIG replacement therapy. Despite receiving immunoglobulin replacement therapy, his condition worsened, with the emergence of autoimmune enteropathy and intermittent thrombocytopenia. His pediatric immunologist noted a lack of response to pneumococcal and tetanus vaccines, prompting further evaluation.
Aidan's symptoms became more severe in his teenage years. He developed recurrent bacterial infections, particularly involving the respiratory tract, and required hospitalization for bacterial pneumonia on two occasions. Chronic sinusitis necessitated multiple courses of antibiotics. In addition, he experienced recurrent viral warts, persistent diarrhea, and progressive weight loss, which was eventually attributed to autoimmune enteropathy.

There was no known family history of primary immunodeficiency, though a paternal uncle had early-onset colitis of unknown etiology.

On physical exam, he appeared thin but otherwise well. His vital signs were stable, with a temperature of 37.2°C, heart rate of 85 bpm, blood pressure of 110/70 mmHg, and oxygen saturation of 98%. Examination of the head and neck revealed pale conjunctiva but no lymphadenopathy. Respiratory examination was notable for bilateral crackles in the lower lung fields. His abdomen was mildly distended with hyperactive bowel sounds. Dermatologic examination revealed numerous viral warts on his hands.

Laboratory Investigations:
A complete blood count revealed a white blood cell count of 3.5 x 10^9/L (4.0-11.0 x 10^9/L), hemoglobin of 115 g/L (130-180 g/L), and platelets of 98 x 10^9/L (150-400 x 10^9/L).

Immunoglobulin levels showed significantly decreased IgG at 2.4 g/L (6.0-16.0 g/L), undetectable IgA at <0.07 g/L (0.8-4.0 g/L), and low IgM at 0.2 g/L (0.5-2.2 g/L).

The Lymphocyte subset flow cytometry demonstrated a marked reduction in CD19+ B cells at 2% (10-20%) and memory B cells at <1% (5-25%). CD4+ T cell count was 450 x 10^6/L (500-1600 x 10^6/L), and CXCR5+ CD4+ follicular T-helper cells were low.
Reviewing his previous vaccine response showed no protective titers to pneumococcal polysaccharide and tetanus toxoid despite prior vaccinations.

Genetic testing revealed a homozygous deletion of a 1,815 base pair region of the ICOS gene (c.126-568.del). This out-of-frame deletion is predicted to lead to a premature stop codon and shortened transcript.

Questions:
1. What is the function of the ICOS in the immune system?
2. How does ICOS contribute to germinal center formation and class-switch recombination?
3. How does the absence of ICOS affect humoral and cellular immunity? Why do patients with ICOS deficiency have low memory B cells and impaired vaccine responses?
4. How does ICOS deficiency lead to both immunodeficiency and autoimmunity?
5. What laboratory tests can be used to assess the functional capacity of T-helper cells in patients suspected of having a primary immunodeficiency?

6. What role do CXCR5+ CD4+ follicular T-helper cells play in humoral immunity, and how does their reduction impact immune function?
7. Compare and contrast ICOS deficiency and CVID. What are the key clinical and immunological differences between ICOS deficiency and classic CVID?

References:

- Schepp J et al. 14 Years after Discovery: Clinical Follow-up on 15 Patients with Inducible Co-Stimulator Deficiency. Front Immunol. 2017 Aug 16;8:964. PMID: 28861081
- Warnatz K et al. Human ICOS deficiency abrogates the germinal center reaction and provides a monogenic model for common variable immunodeficiency. Blood. 2006 Apr 15;107(8):3045-52. Epub 2005 Dec 29. PMID: 16384931.
- Yong PF et al. The role of costimulation in antibody deficiencies: ICOS and common variable immunodeficiency. Immunol Rev. 2009 May;229(1):101-13. PMID: 19426217.