Lab Diagnosis of Immune Defects:

Why Blood is thicker than Water

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• Astellas Canada: Research Grant
Objectives:

(1) Overview of host defenses and relevant immunological diagnostic tests;

(2) Diagnostic strategies: Tailoring Immunology tests to Infectious disease syndromes;

(3) Genetic testing of the MAcrobe: Targeted vs. Agnostic approaches... and why should we even care
Immunology for the ID/MM: Overview of Host Defenses
How to think about Immunity

INFECTIOUS DISEASE

MICROBIOLOGY

Anti-Microbial therapy

HOST
How to think about Immunity

CLINICAL DISEASE (PHENOTYPE)

CLINICAL SUSPICION of Immune Defect

Deficient vs Excessive

Innate vs Adaptive

Inborn vs Acquired
Immunity 101: Why aren’t you sick?

Immune System

INNATE

- Pre-formed
- Pattern Recognition
- Immediate (seconds-hours)

Effectors:
- Complement
- Neutrophils
- Monocytes
- NK cells
- Epithelium
- Endothelium?

ADAPTIVE

- Antigen-induced
- Antigen-recognition
- Delayed (days-weeks)

Effectors:
- Antibody
- B & T lymphocytes

Sense & Search

Ignore / Destroy
Immunodeficiency: Inborn vs Acquired

- **Primary Immunodeficiency (PID)**
- “Primary”: disease–causing genotype
- PID: inborn error of immunity that results in fatal (or life-threatening) infection
- Expanded phenotype:
  - Atopy
  - Immune Dysregulation (Autoimmunity / Auto-inflammatory)
  - Cell proliferation: Lymphoproliferative, Cancer
# Secondary Immunodeficiency (SID)

- “Secondary”: not inherent (genetic)

<table>
<thead>
<tr>
<th>Aging</th>
<th>Iatrogenic immunosuppression (drugs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malnutrition</td>
<td>Infections (e.g. HIV, measles)</td>
</tr>
<tr>
<td>Malignancy</td>
<td>Loss (e.g. Nephrotic; PLE); AutoAbs</td>
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</tbody>
</table>

- Defects; Multifactorial
Microbiology = critical!

**VIRUS**

- RNA: Picorna (e.g. Enterovirus), Orthomyxo, Paramyxo
- DNA: Herpes, HPV

**GRAM (+)**
- Streptococcus, Staphylococcus

**GRAM (-)**
- E. coli, Pseudomonas, Mycobacteria

**FUNGI**
- Yeast: Candida
- Moulds: Aspergillus

**PARASITES**
- Helminths: worms
- Protozoa: Giardia

**PATHOGEN**

- Dictates Immune Defect

*Pneumocystis jiroveci*
DIAGNOSTIC STRATEGIES:
Infections, Infections, everywhere

Meningitis/Encephalitis

Pneumonia

Gastro-enteritis

Cellulitis

Arthritis

UTI
Who’s Got a Problem?

10 Warning Signs of Primary Immunodeficiency

Primary Immunodeficiency (PI) causes children and adults to have infections that are unusually hard to cure. 1:500 persons are affected by one of the known Primary Immunodeficiency (PI) diseases.

If you or someone you know is affected by two or more of the following Warning Signs, consult with a physician about the possible presence of an underlying Primary Immunodeficiency (PI) disease.

1. Four or more new ear infections within one year.
2. Two or more serious sinus infections within one year.
3. Failure of an infant to gain weight or grow normally.
4. Persistent thrush in mouth or fungal infection on skin.
5. Abscesses or recurrences of the same abscess on another area.
6. A family history of PI.
7. Persistent thrush in mouth or fungal infection on skin.
8. Two or more pneumonias within one year.
9. Persistent thrush in mouth or fungal infection on skin.
10. A family history of PI.

Presented as a public service by:

APIQ
Association des Patients Immunodéficients du Québec

Warning signs

- 28 ear infections within 1 year
- 2 pneumonias within 1 year
- Failure to grow normally
- Failure to thrive
- Recurrent deep skin or organ abscesses

Warning signs

- Need for intravenous antibiotics
- Lymphopenia
- Lymphopenia Hypogamma globulinemia

1. "Plus de 2 sinusites par an"
2. "2 infections sévères par an"
3. "Plus de 2 mois de traitement aux antibiotiques par an avec pas d’effets"
4. "Une infection persistante par champignons dans le buche ou sur le pied"
5. "Antécédents familiaux d’un déficit immunitaire primaire"
6. "PLU de 4 citées par an"

These warning signs were developed by the Jeffrey Modell Foundation in consultation with Primary Immunodeficiency experts. For information or referrals, contact the Jeffrey Modell Foundation: info@jmf.org or 1-855-561-4563.
Table 1
The 6 ESID warning signs for adult primary immunodeficiency diseases

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Four or more infections requiring antibiotics within 1 year (otitis, bronchitis, sinusitis, pneumonia)</td>
</tr>
<tr>
<td>2.</td>
<td>Recurring infections or infection requiring prolonged antibiotic therapy</td>
</tr>
<tr>
<td>3.</td>
<td>Two or more severe bacterial infections (osteomyelitis, meningitis, septicemia, cellulitis)</td>
</tr>
<tr>
<td>4.</td>
<td>Two or more radiologically proven pneumonia within 3 years</td>
</tr>
<tr>
<td>5.</td>
<td>Infection with unusual localization or unusual pathogen</td>
</tr>
<tr>
<td>6.</td>
<td>PID in the family</td>
</tr>
</tbody>
</table>

Abbreviations: ESID, European Society of Immunodeficiency; PID, primary immunodeficiency disease.
Who’s Got a Problem?

• **Abnormal frequency of infection**
  - Recurrent Respiratory Tract Infections (RRTIs)
  - Recurrent/recalcitrant warts

• **Abnormal presentation of infections**
  - Fungal CNS infection

• **Specific unusual infections**
  - *Pneumocystis jirovecii* pneumonia
  - *Burkholderia cepacia* complex lymphadenitis
  - Infection with a bug that ID/MM cannot pronounce
The “Immunocompromised” Host

<table>
<thead>
<tr>
<th>Immunosuppressive Diseases or Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Hematologic malignancies (leukemias, lymphomas)</td>
</tr>
<tr>
<td>- Transplantation (bone marrow, solid organ)</td>
</tr>
<tr>
<td>- Acquired immunodeficiency syndrome</td>
</tr>
<tr>
<td>- Neutropenia associated with diseases or drugs</td>
</tr>
<tr>
<td>- Collagen vascular diseases (when treated with immunosuppressive drugs)</td>
</tr>
<tr>
<td>- ICU patients who have had gastrointestinal surgery or perforation, central venous catheter, total parenteral nutrition, broad-spectrum antibiotics, burns, multisystem organ failure, or are neonates</td>
</tr>
<tr>
<td>- Diabetes mellitus with ketoacidosis</td>
</tr>
<tr>
<td>- Iron chelation therapy with deferoxamine</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Immunosuppressive Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Corticosteroids</td>
</tr>
<tr>
<td>- Cancer chemotherapeutic agents</td>
</tr>
<tr>
<td>- Cyclosporine, tacrolimus</td>
</tr>
</tbody>
</table>

USUALLY: Discernable by History, Physical Exam, some basic lab tests
The “Not-Obviously Immunocompromised” Host

**CLINICAL PHENOTYPE**

**MICRO-BIOLOGICAL PHENOTYPE**

**CELLULAR / IMMUNO PHENOTYPE**

**GENOTYPE**

**TESTING**
- targeted
- gene panels
- agnostic

**QUANTITATIVE**
- Too high, too low (consistently so)
- Commonly available
- Fast TAT

**QUALITATIVE**
- Protein detection (flow cytometry; Western blot)
- Functional assays
- Specialized/Research labs
- Slow TAT
In reality: Not always so linear

SYNDROME

IMMUNO-PROFILE

PLEIOTROPIC GENES
Primary immunodeficiency diseases: an update on the classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency

2015: >260 PID (increase of 30 since 2011)
Recurrent Respiratory Tract Infections (RRTIs)

- Recurrent:
- Respiratory:
  - Upper
  - Lower
- Bacterial
- Radiologically documented/clear ance
- Invasive disease
Who let the Pneumococcus out?
Complement:

**CLASSICAL**
- C1q; C1r; C1s
- C4; C2

**ALTERNATIVE**
- C3b;
- Factor D;
- Factor B;
- Properdin

**LECTIN**

Microbe surface

Testing of C’:
1. C3 ; C4
2. Classical C’ + Alternative C’
3. Repeat if ab(n)
   - [R/O in vitro consumption]
   - (+/-) Individual C’ components
   - (+/-) ? Cause
Who let the Pneumococcus out?
### HypoGammaGlobulinemia (HGG)

<table>
<thead>
<tr>
<th>Property</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
<th>IgE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major serum Ig (75%)</td>
<td>Mucosal secretions (breast milk, saliva, tears, mucus)</td>
<td>First type of Ab produced after T cells find an antigen</td>
<td>Usually low levels</td>
<td></td>
</tr>
<tr>
<td>Major extra-vascular Ig</td>
<td>Mucosal secretions (breast milk, saliva, tears, mucus)</td>
<td>First type of Ab produced after T cells find an antigen</td>
<td>Usually low levels</td>
<td></td>
</tr>
<tr>
<td>Crosses placenta</td>
<td>Mucosal secretions (breast milk, saliva, tears, mucus)</td>
<td>First type of Ab produced after T cells find an antigen</td>
<td>Usually low levels</td>
<td></td>
</tr>
<tr>
<td>21 d</td>
<td>6 d</td>
<td>5 – 10 d</td>
<td>2 d</td>
<td></td>
</tr>
<tr>
<td>Monomer</td>
<td>Dimer (secretions)</td>
<td>Pentamer</td>
<td>Monomer</td>
<td></td>
</tr>
<tr>
<td>Fixes complement</td>
<td>Mucosal defences</td>
<td>Fixes complement Agglutination</td>
<td>Bound to mast cells / basophils</td>
<td></td>
</tr>
<tr>
<td>Opsonization</td>
<td>Mucosal defences</td>
<td>Fixes complement Agglutination</td>
<td>Bound to mast cells / basophils</td>
<td></td>
</tr>
</tbody>
</table>

**IgG subclasses:**
- IgG1
- IgG2
- IgG3
- IgG4

**QUALITY:**
1. **SPECIFIC Abs**
   - Vaccines: Tetanus, Hib
   - Natural infections: VZV
2. **DIVERSITY:** SPEP, UPEP, FLC

**RETENTION/LOSS:** ALBUMIN
**B cell Development**

**DEVELOPMENTAL DEFECT**

- HSC
- Pro-B
- Immature B
- Mature B (IgM, IgD)
- Mature B (IgG, IgA, or IgE)
- Plasma Cell
- Memory B

**MATURATION DEFECT**

**LYMPHOPROLIFERATIVE DISORDER**

**TESTING:**
- B cells (CD19/CD20)
- T cells (CD3; CD4; CD8)

**Bone Marrow**

**T cell**
Who let the Pneumococcus out?
NEUTROPHILS:

NEUTROPENIA:
- SEVERE CONGENITAL
- CYCLIC
- many others

LEUKOCYTE ADHESION DEFICIENCY
CD11b/CD18

CHRONIC GRANULOMATOUS DISEASE
DHR-123

NEUTROPHIL COUNT (absolute; manual)

Myeloblast  Promyelocyte  Myelocyte  Metamyelocyte  Band cells  Segmented cells
Who let the Pneumococcus out?
Immunologic w/u for RRTIs:

1) Complement:
   - C3; C4
   - CLASSICAL & ALTERNATIVE C’ Pathways
   - Individual C’ components

2) Immunoglobulins:
   - IgG / A / M / E
   - IgG subclasses
   - Albumin

3) Enumeration of B & T cells

And...

4) Spleen (RRTI + invasive disease)

5) Neutrophils (RRTI + other infections)
   - Quantitative: Abs Neutrophil count
   - Functional: DHR; CD11b/CD18

- PULMONARY / ENT causes:
  - Anatomic
  - Allergic
  - Alpha1-anti-trypsin
  - Primary ciliary dyskinesia (nasal NO)
  - Cystic Fibrosis
  - ENaC (Epithelial Na+ channel disorders):
    - ab(n) Na/Cl in serum;
    - ab(n) sweat Cl-
Relevance of Immunologic w/u:

1) Complement:
   - C3; C4
   - CLASSICAL & ALTERNATIVE C’ Pathways
   - Individual C’ components

2) Immunoglobulins:
   - IgG / A / M / E
   - IgG subclasses
   - Albumin

3) Enumeration of B & T cells

And...

4) Spleen

5) Neutrophils
   - ANC
   - Functional: DHR; CD11b/CD18

VACCINATION against “ENCAPSULATED” organisms

Immunoglobulin replacement

**IVIG or SCIG**
Target IgG: 8-10 g/L

VACCINATION against “ENCAPSULATED” organisms & Early/Self-initiated Abx

G-CSF
BAL showed *Pneumocystis*

Snip excision revealed HPV-6

Culture yielded *Candida albicans*

Pott’s (TB spine)

Kaposi’s sarcoma
Mature B (IgG, IgA, or IgE)

B CELL

T CELL

Proliferation

Cytokines

Cunningham-Rundles, Nat Imm 2005
### Aetiologies of severe combined immunodeficiency

<table>
<thead>
<tr>
<th>Type of SCID</th>
<th>Chromosomal location</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-B^-NK^-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interleukin-7 receptor α-chain deficiency</td>
<td>5p13</td>
<td></td>
</tr>
<tr>
<td>CD3 δ-chain deficiency</td>
<td>11q23</td>
<td></td>
</tr>
<tr>
<td>CD3 ε-chain deficiency</td>
<td>11q23</td>
<td></td>
</tr>
<tr>
<td>T-B^-NK^-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined recessive SCID (γ chain deficiency)</td>
<td>1q31-1q32</td>
<td>6</td>
</tr>
<tr>
<td>RAG1 and RAG2 deficiency</td>
<td>19p13.1</td>
<td>7</td>
</tr>
<tr>
<td>T-B^-NK^-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artemis gene-product deficiency</td>
<td>10p13</td>
<td>8</td>
</tr>
<tr>
<td>RAG1 and RAG2 deficiency</td>
<td>19p13.1</td>
<td>9</td>
</tr>
<tr>
<td>T-B^-NK^-</td>
<td>Adenosine-deaminase deficiency</td>
<td>20q13.11</td>
</tr>
</tbody>
</table>

γc, common cytokine-receptor γ-chain; JAK3, Janus kinase 3; NK, natural killer; RAG, recombination-activating gene; SCID, severe combined immunodeficiency.

**SCID**: NO T cells

**CID (“leaky” SCID)**: LOW/Dysfunctional T cells

**Idiopathic CD4+ lymphopenia**
T cell Defects:

Cunningham-Rundles, Nat Imm 2005
Evaluation of T cells:

- **T / B / NK enumeration:**
  - **T:** CD3$^+$ (total)  
    CD4$^+$ ; CD8$^+$  
    RA$^+$ (naive)/RO$^+$ (memory)
  - **B:** CD19 (or CD20)
  - **NK:** CD3$^-$,16/56$^+$

- **Monocytes:**
  - Manual > Automated
  - CD14$^+$

- **Ig G / A / M / E**

- **TREC:** T cell maturation through thymus

- **TcR repertoire** (PCR; spectratyping): clonality

- **TB-Quantiferon** (or DTH)
Evaluation of T cells:
More Advanced Testing

- **Th17 cells**: Candidiasis
- **Tfh cells**: Hypogammaglobulinemia
- **Treg cells**: Autoimmunity
BUT... WHAT IF IT’S ALL “NORMAL” ??

FUNCTIONAL TESTING
The diagram illustrates the differentiation pathways of T helper (Th) cells into various subtypes:

- **T_{H1}**: Induced by IFN\(\gamma\) and characterized by T-bet expression.
- **T_{H2}**: Induced by IL-4 and characterized by Gata-3 expression.
- **T_{H17}**: Induced by IL-23 and characterized by RoRγt expression.
- **T_{reg}**: Induced by TGF\(\beta\) and characterized by FoxP3 expression.
- **T_{H9}**: Induced by IL-6 and characterized by PU.1 expression.
- **TfH**: Induced by IL-6 and characterized by Bcl-6 expression.

Interleukins (ILs) play a crucial role in these pathways:
- IL-12 activates T_{H1}.
- IL-4 activates T_{H2}.
- IL-23 activates T_{H17}.
- IL-6 activates TfH.
- IL-10 activates T_{reg}.
- IL-9 activates T_{H9}.
- IL-21 activates T_{H1} and T_{H9}.

Activation signals from antigen-presenting cells (APCs) are also indicated.
IL-12 / IFN-γ axis: Mycobacteria; TDF; *Salmonella*

**Monocyte**

- **IFN-γR**
- **IFN-γR1**
- **IFN-γR2**
- **CD40L**
- **IL-12Rβ1**
- **IL-12R**
- **NEMO**
- **STAT1**
- **IL-12**
- **p40**
- **IL-12Rβ1**

**T/NK**

- **IL-1**
- **TNFα**
- **IL-12**
- **IFN-γ**

**Functional Testing... an example**
GENETIC TESTING of the Macrobe (Host)

WHO CARES??

YOU DO!!
Genetic Testing: WHY you should care

• CLINICAL:
  ▪ **MOLECULAR** confirmation of diagnosis
  ▪ Genetic counseling: who else in family is at risk?
  ▪ Prognosis: what complications to monitor

• DIAGNOSTIC:
  ▪ Unambiguous
  ▪ “Forme fruste”
    ▪ variable penetrance; variable expressivity
  ▪ Testing
    ▪ Functional = viable cells [fragile]; Genes = stable

• THERAPEUTIC:
Genetic Testing: WHY you should care

SYNDROME

FUNCTIONAL Immunotherapy:
- IVIG or SCIG
- G-CSF
- Vaccination

HEALTH

MOLECULAR Immunotherapy:
Gene targeting

(Deficient) Cellular phenotype

GENE

DEFICIENT GENES

IMMUNO-PROFILE

PLEIOTROPIC GENE
Genetic Testing: the CARD9 example

Spontaneous CNS candidiasis (*C. albicans*)

Recessive mutations in *CARD9*

qPCR

Cytokine

![Imaging results for CNS candidiasis](image)

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**CSF2**

- mRNA expression (GAPDH)
  - Normal
  - P2

- GM-CSF (ng/mL)
  - Time (h)

---

**CSF2 expression (GUSB)**

- Relative mRNA expression
  - Normal
  - P3
  - P4

---

**GM-CSF**

- Concentrational fluid (CSF) (ng/mL)
  - C. albicans in CSF
  - Normal CSF protein level

---

**Fluconazole**

- Upper limit of laboratory’s reference range for CSF cell count

---

**Tapering**

- Liposomal Amphotericin
  - Surgical resection

---

**Voriconazole**

- Mucor fungus in CSF

---

**Protein (g/L)**

- Upper limit of laboratory’s reference range for CSF protein
Genetic Testing: How?

- **Commercial Labs**
  - Provincial regulations

- **Research Labs**
  - Targeted: 1 gene
  - Panel: Multiple genes
  - Whole gene vs. gene regions

- **Agnostic**
  - “Don’t know what I’m looking for”
  - **Discovery of new genes**

- **Next-generation sequencing**
  - Whole-exome
  - Whole-genome
  - Transcriptome (RNA-seq)

COSTS = Pre-Analytical + Analytical + Post-Analytical
Key Points:

- **Infections (Recurrent / Severe / Unusual)** → THINK OF IMMUNODEFICIENCY (NOT Abx deficiency)

- **Immunodeficiency**: Primary (Genetic) or Secondary

- **Testing**: 
  - Clinical + Microbiological
  - Immunological: **Quantitative** vs. **Qualitative** (Functional)
    - Myeloid: Neutrophils; Monocytes/Macrophages
    - Lymphoid: T / B / NK
    - Humoral: Complement; Immunoglobulins
  - Genetic

- The type of immunodeficiency → Management

- And….
KEEP AN OPEN MIND

IF YOU FIND YOURSELF IN AN AWKWARD PREDICAMENT...

WE ARE JUST A PHONE CALL / EMAIL AWAY & WOULD LOVE TO COLLABORATE WITH YOU

THANK YOU!