Understanding Diagnostic Tests for Immunodeficiency

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What are we testing for?

- Enumerating populations of cells
- Testing cellular functions
- Testing antibody amounts
- Other proteins in the blood
- Genetic testing
- Newborn screening

- Two research projects
# Diagnosis of T cell Diseases

**History, Physical Exam, Lab Tests**

## Table I. Infectious organisms associated with major categories of immune deficiency

<table>
<thead>
<tr>
<th>Organism</th>
<th>Antibody deficiency</th>
<th>Cellular deficiency</th>
<th>Combined deficiency</th>
<th>Phagocyte defect</th>
<th>Complement deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Viruses</strong></td>
<td></td>
<td></td>
<td>All</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Enteroviruses</td>
<td><strong>S typhi</strong></td>
<td>As for antibody deficiency, also: <em>L monocytogenes</em>, <em>S typhi</em>, enteric flora</td>
<td><em>S aureus</em>, enteric flora, <em>P aeruginosa</em>, <em>S typhi</em>, <em>N asteroides</em></td>
<td></td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td><em>S pneumoniae</em>, <em>H influenzae</em>, <em>S aureus</em>, <em>P aeruginosa</em>, <em>C fetus</em>, <em>N meningitidis</em>, <em>M hominis</em>, <em>U ureolyticum</em></td>
<td></td>
<td></td>
<td>As for antibody deficiency, esp <em>N meningitidis</em></td>
<td></td>
</tr>
<tr>
<td><strong>Mycobacteria</strong></td>
<td>No</td>
<td><strong>Nontuberculous</strong> including BCG</td>
<td>Nontuberculous, including BCG</td>
<td>Nontuberculous, including BCG</td>
<td>No</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td>No</td>
<td><em>C albicans</em>, <em>H capsulatum</em>, <em>A fumigatus</em>, <em>C immittis</em></td>
<td>As for cellular deficiency</td>
<td><em>A fumigatus</em>, <em>C albicans</em></td>
<td>No</td>
</tr>
<tr>
<td><strong>Protozoa</strong></td>
<td><em>G lamblia</em></td>
<td></td>
<td><strong>P carinii</strong>, <strong>T gondii</strong></td>
<td><em>P carinii</em></td>
<td>No</td>
</tr>
</tbody>
</table>
History starts before birth

• Find out about family history of immunodeficiencies, early death, consanguinity

• Assess pregnancy, complications (including prematurity)
  – Most IgG is transferred in the 3$^{rd}$ trimester

• Serious primary immunodeficiencies often present after 3-6 months because of maternal antibodies
<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Eight or more new ear infections within 1 year.</td>
<td>Recurrent, deep skin or organ abscesses.</td>
</tr>
<tr>
<td>2</td>
<td>Two or more serious sinus infections within 1 year.</td>
<td>Persistent thrush in mouth or elsewhere on skin, after age 1.</td>
</tr>
<tr>
<td>3</td>
<td>Two or more months on antibiotics with little effect.</td>
<td>Need for intravenous antibiotics to clear infections.</td>
</tr>
<tr>
<td>4</td>
<td>Two or more pneumonias within 1 year.</td>
<td>Two or more deep-seated infections.</td>
</tr>
<tr>
<td>5</td>
<td>Failure of an infant to gain weight or grow normally.</td>
<td>A family history of Primary Immunodeficiency.</td>
</tr>
</tbody>
</table>

Two or more warning signs

Lacking: Autoimmunity, Allergies
Note the histories we don’t get worked too up about

• Strep pharyngitis (even recurrent)
• Superficial skin infections
  – Check the nose, check for MRSA
• Thrush under age 1
  – Especially when bottle fed
• Recurrent viral URIs
  – Think about asthma, day care, school-age siblings (RR 2), lack of breastfeeds (RR 8)
Cellular

Innate

Phagocytic cells
NK cells

Adaptive

T cells

Humoral

Complement

Antibody (B cells)
Counting cells: What does it tell us?

- T cells
  - Subsets (CD4, CD8)
  - Subsets of subsets (nTregs, CD45 isoforms – memory)
  - Subsets of subsets of subsets...
- B cells
  - Memory B cells
- NK cells
  - NKT cells, iNKT cells
- Other cells: neutrophils, eosinophils

- Deficiencies of cell numbers means: failure to generate, failure to survive/maintain, loss
  - Examples: SCID, B cell deficiencies
Immunoglobulin levels vary by age
IgG subclasses also vary by age
B cell function

- Production of antibodies
- Activation of T cells
- Regulation of immune responses

- Immunoglobulin levels
  - IgG$_{1-4}$, IgA$_{1-2}$, IgM, IgE

- Specific antibodies
  - Vaccine responses
  - Responses to specific infections
  - Vaccine challenge (booster)
Memory B cells in CVID

• Warnatz et al, Blood 2002

Table 4. New classification of CVID

<table>
<thead>
<tr>
<th>CVID</th>
<th>Subgroup</th>
<th>No. of patients</th>
<th>CD19+ B cells, % of PBLs</th>
<th>CD27- IgM+ B cells, % of PBLs</th>
<th>CD27+ IgM+/IgD-, % of PBLs</th>
<th>CD27+ IgM-/IgD-, % of PBLs</th>
<th>CD21-, % of B cells</th>
<th>Bryant classification</th>
<th>Splenomegaly</th>
<th>Autoimmunity*</th>
<th>Vaccination†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>a</td>
<td>10</td>
<td>4.9 ± 2.6‡</td>
<td>3.5 ± 1.8</td>
<td>1.2 ± 0.9</td>
<td>0.1 ± 0.1§</td>
<td>44.7 ± 11.0§</td>
<td>A/B</td>
<td>10/10 (100%)</td>
<td>6/10</td>
<td>Neg.</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>13</td>
<td>7.8 ± 3.6</td>
<td>6.5 ± 3.2‡</td>
<td>0.9 ± 0.4‡</td>
<td>0.1 ± 0.1§</td>
<td>9.9 ± 5.7</td>
<td>A/B</td>
<td>5/12 (42%)</td>
<td>6/13</td>
<td>intern.</td>
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<tr>
<td>Group II</td>
<td></td>
<td>7</td>
<td>12.6 ± 4.7</td>
<td></td>
<td></td>
<td>7.6 ± 4.3</td>
<td>3.8 ± 1.9</td>
<td></td>
<td></td>
<td>0.9 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>HD</td>
<td></td>
<td>22</td>
<td>7.7 ± 2.7</td>
<td>4.3 ± 1.6</td>
<td>1.6 ± 1.1</td>
<td>1.6 ± 0.6</td>
<td>7.0 ± 2.7</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
T cell functional testing

• Delayed type hypersensitivity
  – Tests homing, cytokine production

• *In vitro* proliferation
  – Stimulated by mitogens
  – Augmented by cytokines
  – Stimulated by antigens

• Cytokine production

• Receptor signaling pathways
Other serum proteins

- Complement
- Complement inhibitors

Wen et al, JACI 2004
<table>
<thead>
<tr>
<th>Complement components</th>
<th>Deficiency</th>
<th>Gene</th>
<th>Inheritance</th>
<th>Associated features</th>
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<tbody>
<tr>
<td>Classical pathway</td>
<td>C1q</td>
<td>C1Q</td>
<td>AR</td>
<td>SLE, Rheumatoid disease, Infections</td>
</tr>
<tr>
<td></td>
<td>C1r</td>
<td>C1R</td>
<td>AR</td>
<td>SLE, Rheumatoid disease, Infections</td>
</tr>
<tr>
<td></td>
<td>C1s</td>
<td>C1S</td>
<td>AR</td>
<td>SLE, Rheumatoid disease, Infections</td>
</tr>
<tr>
<td></td>
<td>C4</td>
<td>CAA, C4B</td>
<td>AR</td>
<td>SLE, Rheumatoid disease, Infections</td>
</tr>
<tr>
<td></td>
<td>C2</td>
<td>C2</td>
<td>AR</td>
<td>SLE, Vasculitis, Polymyositis, Infections</td>
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<tr>
<td>Lectin pathway</td>
<td>MBL</td>
<td>MBL2</td>
<td>AR</td>
<td>Pyogenic infections</td>
</tr>
<tr>
<td></td>
<td>MASP2</td>
<td>MASP2</td>
<td>AR</td>
<td>Pyogenic infections, SLE</td>
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<tr>
<td>Alternative pathway</td>
<td>Factor D</td>
<td>CFD</td>
<td>AR</td>
<td>Neisserial infections</td>
</tr>
<tr>
<td></td>
<td>Properdin</td>
<td>PFC</td>
<td>XL</td>
<td>Neisserial infections</td>
</tr>
<tr>
<td>C3</td>
<td>C3</td>
<td>C3</td>
<td>AR</td>
<td>Recurrent pyogenic infections, Glomerulonephritis</td>
</tr>
<tr>
<td>Terminal pathway (Membrane attack complex)</td>
<td>C5</td>
<td>C5</td>
<td>AR</td>
<td>Neisserial infections, SLE</td>
</tr>
<tr>
<td></td>
<td>C6</td>
<td>C6</td>
<td>AR</td>
<td>Neisserial infections, SLE</td>
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<tr>
<td></td>
<td>C7</td>
<td>C7</td>
<td>AR</td>
<td>Neisserial infections, SLE, Vasculitis</td>
</tr>
<tr>
<td></td>
<td>C8a</td>
<td>C8α</td>
<td>AR</td>
<td>Neisserial infections, SLE</td>
</tr>
<tr>
<td></td>
<td>C8b</td>
<td>C8β</td>
<td>AR</td>
<td>Neisserial infections, SLE</td>
</tr>
<tr>
<td></td>
<td>C9</td>
<td>C9</td>
<td>AR</td>
<td>Neisserial infections, SLE</td>
</tr>
<tr>
<td>Regulatory proteins</td>
<td>C1 inhibitor</td>
<td>C1INH</td>
<td>AD</td>
<td>Hereditary angioedema</td>
</tr>
<tr>
<td></td>
<td>Factor I</td>
<td>CFI</td>
<td>AR</td>
<td>Recurrent pyogenic infections</td>
</tr>
</tbody>
</table>
|                               | Factor H   | CFH    | AR          | Hemolytic-uremic syndrome, Membranoproliferative
glomerulonephritis                                      |
|                               | CD46       | CD46 (MCP) | AR          | Hemolytic-uremic syndrome, Glomerulonephritis           |
|                               | CD55       | CD55   | AR          | Inab blood group phenotype                              |
|                               | CD59       | CD59   | AR          | Hemolysis pyogenic infections                            |
|                               | CD18       | ITGB2  | AR          | Necrotic lesions, Omphalitis, Leukocyte adhesion
deficiency type 1 (see Sect. 4.4 for more details)      |


aDeficiency implies both complete genetic deficiency and genetic variants (polymorphisms) that predispose to the associated features

bSimilar manifestations are seen with genetic variants of factor I, CD46, CD55, factor B, and C3
What is DNA?

Image adapted from: National Human Genome Research Institute.
What are genes?

Gerstein et al., Genome Research 2007
How do we get genomic DNA?

• Buccal swab

• Immune cells from the blood
Whole exome and genome sequencing

- Targeted sequencing of the protein-coding portion (3%) of the genome
Drawbacks of genetic testing

- Synonymous mutations that affect splicing but not coding
- Intronic mutations
- Undescribed mutations
- Information overload
- Insurers see genetic testing as a form of risk-assessment
- Genetic Non-Discrimination Act of 2009
  - prohibits genetic discrimination in health insurance
  - prohibits genetic discrimination in employment
Undescribed mutations

Rag1

Butte et al, submitted
Intronic mutation example

**A**

*IL7R* genomic DNA
23.2 Kb

Exon 1  2  3  4  5  6  7  8

**B**

*IL7R* cDNA
1.7 Kb

cDNA Number

Exon 1  2  3  4  5  6  7  8

<table>
<thead>
<tr>
<th>1</th>
<th>83</th>
<th>222</th>
<th>380</th>
<th>538</th>
<th>707</th>
<th>801</th>
<th>877</th>
<th>1380</th>
<th>1720</th>
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<tbody>
<tr>
<td>Sig</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>W</td>
<td>TM</td>
<td>B1</td>
<td>S</td>
<td>T</td>
<td>3 UTR</td>
</tr>
</tbody>
</table>

C

*IL7R* Mutations
Inherited by
Proband

**Paternal Mutation**

C to T at CG dinucleotide on anti-coding strand

353 G>A; C118Y

Exon 3 cDNA

222...CTA ACC TGC AAA AAA...379

Translation

L T C K

Dolosorous misense mutation

**Maternal Mutation**

New splice site
Intron 3, cDNA 379(+288) g>a

Genomic DNA

Exon 3

104 bp insert from Intron 3

TGA Stop codon

Premature stop

Decreased expression due to
mRNA degradation

Butte, et al, Clinical Immunology 2007
Ethical Considerations

• Research versus clinical testing

• What is the use of the results?
  – GATTACA

• Testing preadolescent carrier girls?
  – Stigma

• Testing fetuses / blastocysts?
Pre-implantation genetic diagnosis

**PGD for Thalassemia**

1. **DNA is isolated from the removed cell**
2. **PCR is carried out on HBA1 or HBA2 genes using the isolated DNA as a template to produce many copies of the gene.**
   - Primers
   - HBA1
   - Copies of the thalassemia gene
3. **The PCR-amplified DNA is then sequenced**
   - Normal DNA
     - A T C T C A
   - Mutant DNA
     - A T C A C A
4. **The sequence is then compared to a database of known gene sequences to determine whether or not it will cause thalassemia**
Newborn Screening for SCID

Kwan et al, JACI 2013
Take home points

• Functional testing is (often) more useful than enumeration

• Normal ranges for tests vary (normally)
  – By age
  – By context (infection, etc.)
  – By laboratory

• Don’t be afraid to ask your doctor for details of each test
PRIMARY IMMUNE DEFICIENCY RESEARCH IN THE BUTTE LAB (STANFORD)
Point of birth screening for SCID

- Incidence ~1/60,000 births
- Early treatment (Hematopoietic stem cell transplantation) is curative
Current cell counting: Flow Cytometry

- Determining the number of immune cells is a critical factor of diagnosis and treatment of a variety of disorders.
- Limitations: Expensive equipment ($150K), per-test costs high ($500-$1000), requires technical expertise to run, not portable, fluorescent antibodies have a limited shelf life.
- CBC with manual differential: costs $133-$211 / test.
Low-cost, point-of-care tool to count blood cells

- Multiple applications
  - Newborn screening for SCID: count T cells
  - Sepsis screening: count immature neutrophils
  - Immune monitoring: immunodepleting therapies
  - Many other applications
- Glucometer-like / disposable cartridges
- One drop of blood
- Extremely low cost (per test and equipment)
- Battery powered
- Disposable / no maintenance
- Minimal to no training

Garcia, et al., *Biomicrofluidics* 2012;
Counting lymphocytes from whole blood
Identifying Unknown Immune Deficiencies

- Recurrent infections or autoimmunity
- Uncharacterized / unclear diagnosis
- We will examine all major circulating immune cell types at once
  - CD4 and CD8 T cells, B cells, NK cells, monocytes, macrophages, neutrophils, eosinophils, DCs
- Our goal is to study in these patients all major signaling pathways
  - Phospho-flow cytometry screen
  - Complements whole exome/genome sequencing
- Stanford Human Immune Monitoring Core
CyTOF

- Flow cytometer plus time-of-flight mass spectromteter

DVSsciences.com
<table>
<thead>
<tr>
<th>Protein/Marker</th>
<th>Mass</th>
</tr>
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<tbody>
<tr>
<td>CD19</td>
<td>142Nd</td>
</tr>
<tr>
<td>CD8a (Clone RPA-T8)</td>
<td>143Nd</td>
</tr>
<tr>
<td>CD4 (Clone RPA-T4)</td>
<td>144Nd</td>
</tr>
<tr>
<td>CD20 (Clone H1)</td>
<td>145Nd</td>
</tr>
<tr>
<td>CD16* (Clone H1)</td>
<td>146Nd</td>
</tr>
<tr>
<td>CD123</td>
<td>147sm</td>
</tr>
<tr>
<td>CD27</td>
<td>148Nd</td>
</tr>
<tr>
<td>CD45RA</td>
<td>149sm</td>
</tr>
<tr>
<td>CD45 Tot</td>
<td>150Nd</td>
</tr>
<tr>
<td>pp38</td>
<td>151Eu</td>
</tr>
<tr>
<td>CD11c</td>
<td>152sm</td>
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<td>CD14 (M5E2)</td>
<td>153Eu</td>
</tr>
<tr>
<td>IgD</td>
<td>154sm</td>
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<tr>
<td>pERK1/2 (Clone 20A)</td>
<td>156Gd</td>
</tr>
<tr>
<td>IkB tot</td>
<td>157Gd</td>
</tr>
<tr>
<td>pSTAT3</td>
<td>158Gd</td>
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<tr>
<td>pSTAT1</td>
<td>159Tb</td>
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<tr>
<td>pSTAT5</td>
<td>160Gd</td>
</tr>
<tr>
<td>CD33-Er166</td>
<td>161Dy</td>
</tr>
<tr>
<td>CD38</td>
<td>162Dy</td>
</tr>
<tr>
<td>CD24 (Clone ML5)</td>
<td>163Dy</td>
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<td>CD33-Er166</td>
<td>166Er</td>
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<td>CD38</td>
<td>167Er</td>
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<tr>
<td>CD24 (Clone ML5)</td>
<td>168Er</td>
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<td>pSTAT1</td>
<td>169Tm</td>
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<td>CD3</td>
<td>170Er</td>
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<td>CD66 tot</td>
<td>171Yb</td>
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<tr>
<td>pSTAT5</td>
<td>172Yb</td>
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<td>pPLCg2</td>
<td>173Yb</td>
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<tr>
<td>HLADR</td>
<td>174Yb</td>
</tr>
<tr>
<td>CD56</td>
<td>175Lu</td>
</tr>
<tr>
<td>CD25 (M-A251)</td>
<td>176Yb</td>
</tr>
</tbody>
</table>
We’re here to support you.

We need your support to continue cutting-edge research in immune deficiency, infection, and autoimmunity.
• Clinical email for Dr. Butte: mbutte@lpch.org
• Research email: manish.butte@stanford.edu

• If you would like to consider being a benefactor of our research work, please contact the Stanford Office of Medical Development: http://med.stanford.edu/development/