The program will take up to one hour to complete.

This program is designed for primary care physicians.

Other health care professionals working with patients and their families may also find this program of interest.

Gulbu Uzel, MD has disclosed no actual or potential conflict of interest in relation to this educational activity.

During this educational activity Dr. Uzel will not be discussing the use of any commercial or investigational product not approved for any purpose by the FDA.

A lecture about recent discoveries in the study of unusual immune disorders.
Gulbu Uzel, MD  Update on Primary Immunodeficiencies: Recent Discoveries

Program Objectives

Upon completion of this program, participants should be able to:

• Understand the recent discoveries in primary immunodeficiencies especially Job’s syndrome, LAC-1/CGD and SCID
• Identify unusual patients with unusual immune disorders and know how to carry out his/her investigation of patient’s immunodeficiency

Disclaimer

Children’s Hospitals and Clinics of Minnesota accepts no responsibility for the materials presented through these Grand Rounds seminars. Each professional host assumes all responsibility for maintaining confidentiality or obtaining authorization, in accordance with all applicable laws.

Accreditation

Children’s Hospitals and Clinics of Minnesota is accredited by the Minnesota Medical Association to provide continuing medical education for physicians. Children’s Hospitals and Clinics of Minnesota designates this educational activity for a maximum of 1 AMA PRA Category 1 Credits™ toward the AMA Physician’s Recognition Award. Each physician should only claim those credits that he/she actually spent in the activity.

Receiving CME Credit

To receive CME credit you must view the entire program and complete the evaluation form at the end.

Updates on Primary Immunodeficiencies

Gulbu Uzel, MD
Clinical Investigator
Immunopathogenesis Section
Laboratory of Clinical Infectious Diseases, NIAID, NIH
OUTLINE:

UPDATES ON LAD-1
UPDATES ON CGD
THE WORLD REIGNED BY JOB
OTHER FACE OF RAG1/RAG2 DEFECTS

Sibling A
- 5 year old girl
- Omphalitis, perianal abscess in the neonatal period
- History of numerous skin infections, pneumonia and recurrent otitis media
- Frequent hospitalizations in spite of aggressive prophylaxis
- Severe gingivitis, periodontitis

Sibling B
- 3 year old boy
- Omphalitis and elevated WBC in the neonatal period
- Staphylococcal sepsis, typhlitis
- Nonhealing skin ulcer following staphylococcal cellulitis
- Gram negative bacterial arthritis, cellulitis
- Multiple admissions

Clinical features:
- Extreme leukocytosis (15,000 to 70,000/mm³)
- Delayed separation of the umbilical cord, omphalitis
- Severe gingivitis, periodontitis

Flow cytometric analysis of CD18 for SIB A
Surface expression (Unstimulated)

Flow cytometric analysis of CD18 for SIB A
Surface expression (Unstimulated)
Patient 1:
- Caucasian male born to non-consanguineous parents
- Delayed cord separation at 2-1/2 mos
- Pseudomonas sepis at 10 months of age
- Persistent groin ulceration requiring debridement and multiple skin grafts
- Developed colonic and perirectal ulcers
- Matched unrelated bone marrow transplantation at age 21 was complicated by severe graft versus host disease and death.

Case Presentation
Patient 2:
- 20 year old Caucasian female born to non-consanguineous parents
- 10 days of age, developed emphyalis
- Diagnosed with LAD-1 at 9 years of age
- Developed subglottic abscess at age 10 and osteomyelitis of left ankle at age 14
- At age 16 had extensive colitis with perianal fistula formation
- Extensive gingivitis and loss of most permanent teeth

Patient 3:
- 37 year old Caucasian male born to nonconsanguineous parents
- At age 10, presented with poor wound healing after tracheostomy for complicated croup
- At age 18 exploratory laparotomy for presumed appendicitis, diagnosed with Crohn’s Disease
- Recurrent poor healing ulcers on the legs and thighs
- Severe gingivitis and periodontitis, loss of permanent teeth.
- At age 27 emergency laparotomy for ileocecal stenosis resulted in right hemicolectomy
- At age 35, he was started on infliximab for LAD-1 associated colitis

CD18 expression in circulating WBCs

<table>
<thead>
<tr>
<th>CD18</th>
<th>CD3+</th>
<th>CD4+</th>
<th>CD57+</th>
<th>TCR αβ+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in</td>
<td>in</td>
<td>in</td>
<td>in</td>
</tr>
<tr>
<td>PATIENT 1</td>
<td>5.3</td>
<td>8.4</td>
<td>57.4</td>
<td>98.9%</td>
</tr>
<tr>
<td>PATIENT 2</td>
<td>10.6</td>
<td>28.3</td>
<td>20.0%</td>
<td>5.5%</td>
</tr>
<tr>
<td>PATIENT 3</td>
<td>18.8</td>
<td>5.2</td>
<td>7.0%</td>
<td>5.5%</td>
</tr>
</tbody>
</table>

WHERE DO THE CD 18+ LYMPHOCYTES COME FROM?
- Are they maternal?
- Could they be transfusion related?
- Is this a reversion/mosaicism?

- 98.9% of the CD18+ cells were TCR αβ+
- 1% and 5% of all the CD18+ cells from patients 1 and 2 were CD3-/CD56+/CD16+
- The CD18+ cells were also perforin+, consistent with their cytotoxic potential.
Microsatellite assays were run for CD3+/CD18+ vs CD3+/CD18- cells from 3 patients. Seven distinct microsatellite sequences were amplified by the PCR, data assessed for unique microsatellite polymorphisms. Identical patterns for the CD18+ and CD18- lymphocytes: CD18+ Cells originate from the patient.

RESULTS:

We have identified three adult LAD-1 patients with somatic mosaicism who survived into adulthood without allogeneic bone marrow transplants. Allelic change was observed only in the genomic DNA from a small subset of circulating CD8+ T cells. Microsatellite analyses proved that the lymphocytes carrying both the mutant and revertant CD18 alleles were endogenous in each patient. In two patients with different homozygous missense mutations, reversion was not to the wild-type but to a third amino acid. In contrast to reports in patients with immunodeficiency, our patients 1 and 2 had novel reversions that partially or fully restored the function but were not WT. It is the reversion to relatively normal function that is important in the expansion of these cells, and not the reversion to WT sequence.
Spontaneous reversion of mutations are more valuable if the corrected cells acquire a selective survival or growth advantage. A similar survival or growth advantage may have helped these long-lived memory effector T cells populate and persist in the peripheral blood in LAD-1.

The limitation of reversion mutations to subsets of cytotoxic lymphocytes in LAD-1 suggests that the cytotoxic T cells have some predisposition to mutation through their propensity for DNA rearrangement and to positive selection, possibly through their anti-infective capacity.

Inflammatory bowel disease in all three patients leaves open the possibility that these cells may be disadvantageous to the host.

We are unable to determine the earliest time point these cells were detectable in peripheral blood since we do not have PBMCs dating as early as birth or infancy.

Whether these reversion mutations have any effect on patient survival remains to be determined.

Could these reversions be the cause or consequence of long-term survival in this severe immunodeficiency?

**Chronic Granulomatous Disease (X, AR)**

- Recurrent life-threatening infections with catalase-positive bacteria and fungi and tissue granuloma formation
- Infections: pulmonary, cutaneous, lymphatic, hepatic, bone
- Bacteremia rare

**Frequent offenders (5 most common):**

- S. aureus
- S. marcescens
- B. cepacia
- Nocardia spp.
- Aspergillus spp

**Others:**

- Chromobacterium violaceum
- Paecilomyces spp.
- Granulobacter bethesdensis

We are unable to determine the earliest time point these cells were detectable in peripheral blood since we do not have PBMCs dating as early as birth or infancy.

**Diagnosis of Chronic Granulomatous Disease (X, AR)**

**Dx:**

1. PMN nitroblue tetrazolium reduction (NBT)
2. PMN dihydrorhodamine 123 oxidation (FACS)
3. Chemiluminescence, Staph killing

**Diagnosis of CGD: DHR Assay**

**Normal**

**gp91-phox deficient CGD (X-linked)**

**X-linked CGD carrier**
**Genetics of Chronic Granulomatous Disease (X, AR)**

X-linked, chr. Xp21 (70% of cases)
- Defect in gp91phox
- Carrier females are mosaic (Lyonization)
- About 1/3 of carriers are sporadic, from sperm

Autosomal recessive (30% of cases)
- Defect in p47phox: 20-30% (Chr 7)
- Defect in p67phox: <5% (Chr 1)
- Defect in p22phox: <5% (Chr16)

1/2000 carry the gene for the most common AR form

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**27 year old woman**

Referred from her internist for evaluation of recurrent cutaneous boils with *S. aureus* and an IgE of 12,376 IU. “Bronchitis and sinusitis at least once a year” and persistent eczema requiring topical steroids. Never been hospitalized but is having “more trouble” lately.

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**Hyper IgE Syndrome (HIES)**

- Autosomal Dominant disorder, formerly known as Job's Syndrome
- Unidentified in 2007
- 
  - Elevated serum IgE levels.
  - Eosinophilia.
  - Dermatitis.
  - Skeletal abnormalities (scoliosis, lack of primary teeth shedding, and recurrent fractures).
  - Staphylococcal skin infections.
  - Lung cysts.
  - Recurrent infections.
Gulbu Uzel, MD  Update on Primary Immunodeficiencies: Recent Discoveries

RCA

Ahmed Gharib MD, Roderic Pettigrew, PhD, MD

<table>
<thead>
<tr>
<th>DNA Binding</th>
<th>SH2 Domain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1144 G&gt;A;</td>
<td>1181 C&gt;G;</td>
</tr>
<tr>
<td>R130Q</td>
<td>R145S</td>
</tr>
<tr>
<td>1150 T&gt;C;</td>
<td>1381 G&gt;A;</td>
</tr>
<tr>
<td>F384L</td>
<td>V461L</td>
</tr>
<tr>
<td>1288 G&gt;A;</td>
<td>1387 delGTG;</td>
</tr>
<tr>
<td>R423Q</td>
<td>V463del</td>
</tr>
<tr>
<td>1381 G&gt;C;</td>
<td>1393 T&gt;G;</td>
</tr>
<tr>
<td>R423Q</td>
<td>S465A</td>
</tr>
<tr>
<td>1832 G&gt;A;</td>
<td>1861 T&gt;G;</td>
</tr>
<tr>
<td>S611N</td>
<td>F621V</td>
</tr>
<tr>
<td>1865 C&gt;T;</td>
<td>1872 C&gt;T;</td>
</tr>
<tr>
<td>F621V</td>
<td>V637M</td>
</tr>
<tr>
<td>1909 G&gt;A;</td>
<td>1915 C&gt;G;</td>
</tr>
<tr>
<td>V637M</td>
<td>P639A</td>
</tr>
<tr>
<td>1939 A&gt;G;</td>
<td>1954 G&gt;A;</td>
</tr>
<tr>
<td>N647D</td>
<td>R652C</td>
</tr>
</tbody>
</table>

Signal Transducer and Activator of Transcription (STAT)

Family of 6 highly conserved proteins
Get signal from receptor complex to nucleus
Bind to each other and to other proteins
Bind to DNA
Turn on or off specific genes

STAT3

Key mediator for many pathways
including those of the immune system, cancer, wound healing, vascular remodeling. Expressed widely in most tissue types.
**Update on Primary Immunodeficiencies: Recent Discoveries**

**STAT3**
- IL-6
- IL-10
- TGFβ
- MCP-1

**IL-12**
- TNFα
- IFNγ
- IFNβ

**IL-10**
- gp130

1. **R382Q**
2. **6**
3. **67**

**1145 G-A R382Q Hotspot: Somatic Mosaic**

- **WildType G**
- **Homozygous G/G**
- **Heterozygous G/A**
- **Lymphocytes Mosaic G/A**
- **Buccal swab Mosaic G/A**

**Absence of Th17 cells**
- **Overnight SEB Stimulation**
- **Gated on CD4+**

**IL-17 Drives Inflammation and Killing**

**Matsuzaki, Umemura Microbiol Immunol 51:1139, 2007**

**Control osteoclasts**
- Patients with HIES have increased number of bone fractures

**HIES osteoclasts**

**Matsuzaki**, *Nature*, 2008

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*Children's CME 1-8-2009*
HIES

- Stay tuned: more molecular work being done
- Mosaic patient may be critical to help explain pathophysiology

Patient 1:
5 year old Caucasian male referred for immune evaluation

Medical history significant for:
- Evans syndrome at 10 months (Treated with steroids, IVIG, cyclosporine, vincristine)
- Neutropenia at 20 months
- Guillain-Barre at 2 ½ years (treated with IVIG)
- Persistent hemolytic anemia, thrombocytopenia and neutropenia at 3 years (received 4 doses of Rituximab combined with steroids and cyclosporin)
- Psoriasis
- Vitiligo at 4 years

Infectious Disease hx:
- 2 recent pneumonias, bronchoscopy revealed H. influenza
- No opportunistic infections
- Nonspecific colitis, bacterial overgrowth

Chest CT: mild bronchiectasis scarring from previous infections
LABS: Low IgG level at 4 years (IgG: 391, normal IgA, normal IgM, IgE undetectable), Protective diptheria and tetanus titer, unprotective pneumococcal titer.
He was started on IVIG q4weeks

LABS: WBC: 3.5K, 4% Eos
CD3+/CD4+: 115/mm3
CD3+/CD8+: 800/mm3
CD19+: 120/mm3
CD3-/CD16CD56: 100/mm3
CD3+HAL-DR+: 12%

Mitogen stimulation: significantly low proliferation
TCR VB: all TCR VB families represented
TRECS: very low copy numbers

<table>
<thead>
<tr>
<th>Disease</th>
<th>Relative frequency</th>
<th>Inheritance</th>
<th>Cells affected</th>
<th>Gene Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticular dysgenesis</td>
<td>&lt;1%</td>
<td>AR</td>
<td>T, B, NK, PLTS</td>
<td>?</td>
</tr>
<tr>
<td>Alymphocytosis</td>
<td>10%</td>
<td>AR</td>
<td>T, B</td>
<td>RAG1, RAG2</td>
</tr>
<tr>
<td>Absence of T lymphocytes</td>
<td>50%</td>
<td>XL</td>
<td>T, NK</td>
<td>yc chain</td>
</tr>
<tr>
<td>ADA deficiency</td>
<td>20%</td>
<td>AR</td>
<td>T, B, NK</td>
<td>ADA</td>
</tr>
</tbody>
</table>

Mutations in recombination activating genes 1 and 2 (RAG1 and RAG2): cause a spectrum of severe immunodeficiencies
Classical (T(-)B(-)SCID) and Omenn syndrome (OS)
Increasing number of peculiar cases.
Hypomorphic mutations leading to reduced V(D)J recombination may lead to autoimmunity while lacking the characteristic features of Omenn syndrome and maintaining the ability to form specific antibodies

SEQUENCING OF RAG1 AND RAG2:
Compound heterozygote:
M435V (paternal allele) and R699W (maternal allele)
The Clinical spectrum of hypomorphic RAG mutations.

Patient 2:
- Disseminated M. avium complex infection with hepatosplenomegaly, persistent sterile granulomatous skin lesions, and diarrhea.
- Low T and B cells with elevated NK cells.
- Anormal lymph node and white spleen architecture and had a fatty thymus without Hassel's corpuscles.
- She was compound heterozygous for two RAG1 mutations (R396C, R975Q) previously reported in Omenn syndrome.

Patient 3:
- History of multiple severe bacterial pneumonias, herpetic gingivitis, and zoster after age 2.
- Absent naïve T cells and normal levels of other lymphocytes. Normal immunoglobulins with normal specific antibody titers.
- She was compound heterozygous for RAG1 R474C and del256-7 causing K86fs.

These distinct presentations of hypomorphic RAG mutations highlight the diverse role of RAG in immune protection and autoimmunity. Recognizing patients with these RAG mutations is key to earlier diagnosis and treatment, and improving patient survival.