Autoimmune Lymphoproliferative Syndrome (ALPS):
An Evolving Story

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Autoimmune Lymphoproliferative Syndrome (ALPS) Findings

Lymphadenopathy

CD3+ T cells

Adult Control

α/β TCR DNT = 0.2% (5/mm3) [nl ≤ 1.5%]

ALPS Patient

Alpha-beta TCR DNT = 33.7% (1071/mm3)
Autoimmune Lymphoproliferative Syndrome (ALPS)

• Diagnostic triad
  ▪ Non-malignant lymphadenopathy
  ▪ Increased level of $\alpha/\beta$ DNT cells (>1.5% lymphs)
  ▪ Defective in vitro lymphocyte apoptosis or FAS mutation associated with ALPS

• Additional features (not used for the diagnosis)
  ▪ Autoimmune disease usually involves blood cells
  ▪ Typical histological findings in lymphatic tissue
  ▪ Family members with similar clinical findings
  ▪ History of lymphoma in the family
Revised Classification System for ALPS
Blood 116:35-40, 2010

• ALPS-FAS: FAS mutation
• ALPS-sFAS: somatic FAS mutation
• ALPS-FASL: FASL mutation
• ALPS-CASP: CASP10 mutation
• ALPS-U: unknown basis for ALPS
• ALPS-like: clinical phenotype, normal DNTs and/or normal FAS kill assay
Model for Dominant Negative Effect of Fas Mutation

1/8 WT Fas Trimer

Active Signaling Complex

Mixed WT/Mutant Fas Trimer

7/8

APOPTOSIS

DISC

NO APOPTOSIS

≥1 mutant Fas protein in the trimer prevents formation of the active signaling complex: inhibits Fas mediated apoptosis
The Genetics of ALPS–FAS Is More Complex

- About 2/3s of ALPS–FAS patients have mutations affecting either the death domain (exon 9) or the transmembrane/ intracellular domains (exons 6–8)

- However about 1/3 of ALPS–FAS patients have extracellular mutations and a less severe clinical phenotype
Summary of the Extracellular FAS Mutation Findings

- ~70% involve nonsense, splice site or insertion/deletion with frameshift
- Most extracellular mutations result in low FAS expression and nonsense mediated RNA decay or protein instability
- The defect could be corrected by wild type FAS overexpression
- Haploinsufficiency explains some ALPS
ALPS Associated with Somatic FAS Mutations

- 31 pts who had clinical and lab findings of ALPS (DN'Ts with FAS mediated apoptosis is less affected) and normal genomic FAS sequencing
- Evaluation based on purifying DNTs for FAS sequencing
- 12 of 31 were found to have somatic FAS mutations (affecting intracellular domains)
Biomarkers in ALPS
ALPS–FAS=162, sFAS=9, family=94, unknown=52

- Clinically looks like ALPS and DNT >1%:
  - FAS mutation not likely (<10%):
    - sFasL <300 pg/ml (none so far)
    - DNT 1–2% and B12 <1000 ng/L or IL–10 <20 pg/ml
  - FAS mutation very likely (>90%):
    - DNT >2% and sFasL >300 pg/ml
    - DNT >4% and IL–10 >40 pg/ml or B12>1500 ng/L

- If germline FAS is normal and +biomarkers, evaluate for a somatic FAS mutation
Lymphoma in ALPS

• Total of 17 ALPS patients in NIH ALPS 1a cohort have developed either HL or NHL
  – 16 ALPS-FAS (n=173)
  – 1 ALPS-U
  – 7 Hodgkin and 10 Non-Hodgkin Lymphoma

• Published data (Blood, 98:194-200, 2001)
  – Malignant B cells had heterozygous Fas mutation
  – RR for HL is 51 fold and NHL is 14 fold
  – Mean age lymphoma diagnosis ~23 yrs and tumors respond to conventional therapy
Summary of the Autoimmune Lymphoproliferative Syndrome

- Demonstrates role of Fas mediated apoptosis:
  - Lymphocyte homeostasis - lymphoproliferation
  - Eliminating specific auto-reactive cells - autoimmunity
  - Eliminating lymphocytes with potential for malignant transformation - increased risk for lymphoma
- Additional factor(s) contribute to autoimmunity
- ALPS-U: many are sporadic cases, suggest other genetic and/or environmental causes
- Somatic Fas mutations raise additional questions
Apoptosis Pathways

Extrinsic
- FASL
- FAS
- Caspase 10

Intrinsic
- Cytokine withdrawal
- Chemotherapy
- DNA damage: x-ray
- BCL2 family

Effector Caspases (3/7)

APOPTOSIS
Patient 58: Defect in Cytokine Withdrawal Induced Apoptosis

Normal Fas mediated (extrinsic) apoptosis

Anti-Fas

Cytokine (IL-2) withdrawal

Abnormal cytokine (IL-2) withdrawal induced (intrinsic) apoptosis

Staurosporine

γ-Irradiation

Cell survival (%) vs. Dose (ng/ml)

Cell survival (%) vs. Dose (nM)

Cell survival (%) vs. Time (days)

Cell survival (%) vs. Dose (x10^2 rads)
Low BIM Levels in Pt 58 Are Associated with a Mutation in NRAS

Expression Microarray: NRAS pathway likely affected

Sequencing Data

38G>A

Normal donor

Lymphoblasts

Monocytes

Epithelial cells

Heterozygous NRAS mutation (G13D) - gain of function in NRAS

Proc N Acad Sci USA 104:8953-8, 2007
Somatic Gain of Function Mutation in KRAS

Blood 2010
Impaired Intrinsic Apoptosis

FAS Apoptosis

IL-2 Withdrawal
## Summary RAS Mutation Pts

<table>
<thead>
<tr>
<th>Patient/gene</th>
<th>Lymphadenopathy</th>
<th>HSM</th>
<th>↑DNTs</th>
<th>Autoimmunity</th>
<th>Granulocytosis/Monocytosis</th>
<th>B-Cell lymphocytosis</th>
<th>Apoptosis defect</th>
<th>Lymph Node Histopathology</th>
<th>Specific Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>P58 - NRAS G13D</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+/-(−2%) direct antoglobulin test, ANA, rheumatoid factor/anti-phospholipid abs</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>No DNTs/plasmacytosis</td>
<td>Transient cryoglobulinemia vasculitis; Cutaneous B-cell lymphoma</td>
</tr>
<tr>
<td>P260- NRAS G13D*</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>HA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>No DNTs/granulocytes</td>
<td>JMML-like</td>
</tr>
<tr>
<td>NRAS Q61P*</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Severe ITP/colitis</td>
<td>+</td>
<td>+</td>
<td>N.D.</td>
<td>No DNTs/granulocytes</td>
<td>JMML-like; BMT at the age of 8y</td>
</tr>
<tr>
<td>KRAS G13C</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>HA/ITP/ANA/RF/anti-phospholipid abs</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>No DNTs/plasmacytosis</td>
<td>Recurrent infections; Mild Factor XI deficiency</td>
</tr>
<tr>
<td>KRAS G12D</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>anti-neutrophil/anti-platelet/anti-phospholipid abs</td>
<td>+</td>
<td>+</td>
<td>N.D.</td>
<td>N.D.</td>
<td>Cutaneous vasculopathy (presumed)</td>
</tr>
</tbody>
</table>

### Leukemias: AML, JMML, CMML
RALD—RAS–Associated Autoimmune Leukoproliferative Disorder

Similarities among NRAS and KRAS pts

- Accumulation of lymphoid and myeloid cells
  - splenomegaly/lymphadenopathy, B cell lymphocytosis, relative or absolute monocytosis
- Immune dysregulation: autoimmunity and autoantibodies
- May overlap with JMML in infancy
- DNTs and biomarkers generally normal
Lessons Learned by Experiments of Nature Resulting in Autoimmunity

• Specific gene defects that impact peripheral tolerance associated with lymphocyte apoptosis can result in increased risk for autoimmunity
• Phenotypic variability point to the complexity in the evolution of autoimmune processes
• The maintenance of peripheral tolerance (and the development of central tolerance) are complex and involve multiple mechanisms
• There may be a hierarchy of mechanisms that maintain self tolerance to various tissues
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