Severe combined immunodeficiencies

DESPINA MOSHOUS

Severe combined immunodeficiencies clinical aspects

- first clinical signs early, in general < 6 months
- without treatment, lethal in the first year of life
- bacterial, fungal, viral infections
- opportunistic pathogens
- recurrent & therapy resistant infections
- profound / disseminated Candida infections
- interstitial pneumopathy (pneumocystis jirovecii +++)
- persisting or recurrent diarrhoea
- denutrition ++
- persisting viral infection (respiratory virus, CMV)

• absence of lymphoid tissue
• absence of thymus
• generalised erythodermia
• disseminated BCGitis
• anamnesis: positive family history ?
• consanguinity ?
• lymphopenia +++
• hypogammaglobulinemia

Severe combined immunodeficiency T-B-NK+

• absence of T and B lymphocytes
• presence of NK cells
• lethal in the first year of life
• 20% of human SCID
• autosomal recessive transmission
• curative treatment BMT

Lymphoid differentiation

Ly NK

T γδ³

T CD8

T CD4

IgG, A, E

IgM

T-B-SCID
Two types of human T-B-SCID

Radioresistant T-B-RR

Radioresitive T-B-RS

deficiency in Rag 1 or 2

Molecular analysis of V(D)J recombination
**V(D)J recombination assay**

![Diagram of V(D)J recombination assay](image)

**Analysis of the first steps of V(D)J recombination**

"radioresistant" T-B-SCID (RR-SCID)

**Defect of the V(D)J recombination in RR-SCID cells**

![Diagram showing defect in RR-SCID cells](image)

**Analysis of the later steps of V(D)J recombination**

"radiosensitive" T-B-SCID (RS-SCID)

**Defect of the V(D)J recombination in RS-SCID cells**

![Diagram showing defect in RS-SCID cells](image)

**Defect in T and B cell development**
**Defect in V(D)J Recombination (CJ>SJ)**
**Radiosensitivity to γ rays**
**Defect in DNA-dsb Repair**
**Mutation in the DNA-PKcs gene**

- Defect in T and B cell development
- Defect in V(D)J Recombination (CJ>SJ)
- Radiosensitivity to γ rays
- Defect in DNA-dsb Repair
- Mutation in the DNA-PKcs gene
ubiquitous DNA repair/recombination machinery

NHEJ

The Gene for Severe Combined Immunodeficiency Disease in Athabascan-Speaking Native Americans Is Located on Chromosome 10p

- Navajo, Apache Native American Indians
- Autosomal recessive transmission
- Severe lymphopenia (T and B) infections
- Treatment BMT
- Localization Chromosome 10p

RS-SCID/A-SCID

same phenotype

similar localization

no candidate genes
Cloning of Artemis

Artemis is ubiquitously expressed

Artemis nuclear localization

Artemis complements the RS-SCID V(D)J recombination defect

Artemis complements the radiosensitivity of RS-SCID fibroblasts

Artemis is mutated in human RS-SCID
atypical forms of « radiosensitive » T−B− SCID

**Jean-Pierre de Villartay**
- Barbara Corneo
- Régine de Chasseval
- Nathalie Nicolas

**INSERM U429**

**Alain Fischer**
- Françoise Le Deist
- Marina Cavazzana-Calvo
- Nada Jabado

• Isabelle Callebaut (CNRS, Paris)
• Marton J. Cswan (UCSF)
• Lanying Li
• Steve Jackson (Welcome/CRC)
• Nicholas J. Finnie
• Dora Papadopoulo (Curie, Paris)
• Noel Philippe (Debrousse, Lyon)
• Ozden Sanal, Ilhan Tezcan, (Hacettepe U., Turkey)
• Sanger Center (UK)

atypical RS-SCID

**lymphoid specific**

RR T−B−-SCID
defect in RAG1 or RAG2

**ubiquitous DNA repair/recombination machinery**

RS T−B−-SCID
defect in Artemis

### intrauterine growth delay

<table>
<thead>
<tr>
<th>at birth</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>head circumference</td>
<td>32 cm (&lt;3 P)</td>
<td>32.5 cm (3-10 P)</td>
<td>33 cm (3-10 P)</td>
</tr>
<tr>
<td>height</td>
<td>48 cm (3-10 P)</td>
<td>49 cm (3-10 P)</td>
<td>47 cm (3 P)</td>
</tr>
<tr>
<td>weight</td>
<td>2860 g (3-10 P)</td>
<td>3260 g (10-25 P)</td>
<td>2830 g (10-25 P)</td>
</tr>
</tbody>
</table>

parents’ head circumference: mother: 50-90 P father: 90 P

### atypical RS-SCID (µSCID)

- **P1** - died at the age of 11 months (interstitial pneumonia)
- **P2** - admission at 16 m: repeated infections, failure to thrive, severe growth delay, hypogammaglobulinemia (IgG and IgA)
- **P3** - admission in the age of 1.5 months, 600 lympho,
- **P4** - atypical RS-SCID
**V(D)J recombination**

*in vivo*
- normal proliferation upon PHA-stimulation
- diversified Vbeta repertoire (P2 and P3)
- normal TCR junctions (presence of \(\alpha\beta\) and \(\gamma\delta\) T)
- but progressive T and B lymphopenia

*in vitro*
- normal frequency of cj and sj-formation
- imprecise signal joint formation

**Jean-Pierre de Villartay**
INSERM U429

- Barbara Corneo
- Régina de Chasseval
- Dietke Buck

**DNA Ligase IV mutations in siblings with atypical RS SCID (\(\mu\)SCID)**

**Jean-Pierre de Villartay**
INSERM U429

- Barbara Corneo
- Régina de Chasseval
- Dietke Buck

**M.R. Lieber**
Norris Comprehensive Cancer Center, LA, California, USA

**Alain Fischer**
- Françoise Le Deist
- Marina Cavazzana-Calvo
- J.-L. Casanova

**clinical manifestations**
**V(D)J recombination defects**

Nature of the mutation

- V(D)J activity
  - completely abolished
  - residual activity
  - "typical" T-B-SCID
  - ?

**Omenn Syndrome**

Severe combined immunodeficiency with diffuse and exudative erythroderma, lymphadenopathy, hepatosplenomegaly, protracted diarrhea, failure to thrive

- low IgM, IgA, IgG but hyper-IgE
- absence of peripheral B cells,
- hypereosinophilia
- hyperlymphocytosis T with activated T cells infiltrating tissues (skin, gut)
- restricted heterogeneity of T cell repertoire, oligoclonality

**T-B-SCID and Omenn Syndrome in the same family**

<table>
<thead>
<tr>
<th>I</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omenn</td>
<td>T-B-SCID</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T cells</th>
<th>B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>6,300</td>
<td>0</td>
</tr>
<tr>
<td>1,900</td>
<td>180</td>
</tr>
</tbody>
</table>


**Mutation in RAG2 (R39G/R229Q)**

**What makes a T-B-SCID develop a Omenn syndrome?**

- residual activity generates some lymphocyte clones

- no stimulation
- stimulation by a second factor

- no/few circulating lymphocytes
- clonal expansion of lymphocytes

**T-/-; B- SCID**
Omenn syndrome = leaky SCID

- "historically" defect in Rag1 and Rag2,
- but also mutations in
  - ARTEMIS
  - DNA ligase 4
  - RNA component of mitochondrial RNA processing endoribonuclease
  - adenosine deaminase
  - IL-2 receptor gamma
  - IL-7 receptor alpha
  - CHD7....

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Immunotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAG1/RAG2</td>
<td>T+ B- NK-</td>
<td>SCID</td>
</tr>
<tr>
<td>Artemis</td>
<td>T+ B- NK-</td>
<td>SCID</td>
</tr>
<tr>
<td>ADA</td>
<td>T+ B- NK-</td>
<td>SCID, multisystem involvement</td>
</tr>
<tr>
<td>DNA ligase 4</td>
<td>T+ B- NK-</td>
<td>SCID, microcephaly</td>
</tr>
<tr>
<td>RMRP</td>
<td>T+ B- NK-</td>
<td>CHH, SCID</td>
</tr>
<tr>
<td>IL-2 receptor γ</td>
<td>T+ B- NK-</td>
<td>SCID</td>
</tr>
<tr>
<td>IL-7 receptor α</td>
<td>T+ B- NK-</td>
<td>SCID</td>
</tr>
<tr>
<td>22q11</td>
<td>T+ B- NK+</td>
<td>DiGeorge syndrome</td>
</tr>
<tr>
<td>CHD7</td>
<td>T- B+ NK+</td>
<td>CHARGE syndrome</td>
</tr>
</tbody>
</table>

a particular form of RS-SCID

- repeated pulmonary infections
- severe T and B lymphocytopenia
- hypogammaglobulinemia (IgG & IgA)
- polyclonal T and B cell populations
- radiosensitivity
- chromosomal anomalies
- phenotype resembling the immune deficiency in Ataxia teleangiectasia (AT) and NBS syndrome

1mo. 2.5 y.

P68:
- candidiasis and protracted diarrhea soon after birth
- lymphadenopathies at age of 9 mo.
- nodular lung infiltrate
- hypogammaglobulinemia
- autoimmune anemia, thrombocytopenia
- severe lymphocytopenia

- lymphoproliferation, treated by anti-B cell specific AB,
- patient died 5 days after diagnosis
- massive infiltrations of lung, liver, skeletal muscle (autopsy)

Clinical presentation

87% of patients display wasting 10 Gy

10 Gy

0 Gy
clinical presentation

P69:
- candidiasis, recurrent respiratory and pulmonary infections soon after birth
- at age of 10 months detection of:
  - severe lymphocytopenia
  - hypogammaglobulinemia
  - intravenous Ig treatment
  - BMT (10/10 MUD) at age of 5 years
  - B cell lymphoma of recipient origin localized to the liver day+38 post BMT
  - death 12 days later despite anti-B ab

P70:
- recurrent otitis and bronchopneumonia from 1 year of age
- bronchiectasis
- at age of 11 years cerebral abcess (Toxoplasma gondii)
- died at age of 13 years because of sepsis and respiratory failure

clinical presentation – family2

P72:
- recurrent pulmonary infections
- bronchiectasis at 4 years of age
- failure to thrive
- at 7 years: cholangitis, liver disease and protracted diarrhea caused by Cryptosporidium infection
- fatal liver cirrhosis leading to death at age of 16 years

immunological data

<table>
<thead>
<tr>
<th>Patients</th>
<th>P68</th>
<th>P69</th>
<th>P70</th>
<th>P72</th>
<th>Normal values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes/µl</td>
<td>64</td>
<td>500-1,100</td>
<td>155-610</td>
<td>378-746</td>
<td>2,500-3,500</td>
</tr>
<tr>
<td>CD3</td>
<td>8</td>
<td>250-580</td>
<td>77-321</td>
<td>200-350</td>
<td>1,500-2,500</td>
</tr>
<tr>
<td>CD4</td>
<td>14</td>
<td>145-341</td>
<td>21-133</td>
<td>114-185</td>
<td>900-2,000</td>
</tr>
<tr>
<td>CD8</td>
<td>12</td>
<td>90-290</td>
<td>57-232</td>
<td>85-115</td>
<td>400-1,000</td>
</tr>
<tr>
<td>CD19</td>
<td>8</td>
<td>8-110</td>
<td>0-5</td>
<td>0-51</td>
<td>200-600</td>
</tr>
<tr>
<td>CD16/CD56</td>
<td>18</td>
<td>227</td>
<td>90</td>
<td>70</td>
<td>100-500</td>
</tr>
<tr>
<td>PHA cpmp x 10³</td>
<td>35.8</td>
<td>26.6 ± 22</td>
<td>16.8 ± 14.6</td>
<td>15.8 ± 22</td>
<td>&gt;40</td>
</tr>
<tr>
<td>Tetanus toxoid</td>
<td>31.9</td>
<td>10.0 ± 12.6</td>
<td>3.5 ± 3.9</td>
<td>59.8 ± 32</td>
<td>&gt;15</td>
</tr>
<tr>
<td>IgG (g/l)</td>
<td>14.4 (9 m)</td>
<td>1.33 (10 m)</td>
<td>2.91 (5 yrs)</td>
<td>2.42 (8 yrs)</td>
<td>4.2-12</td>
</tr>
<tr>
<td>IgA (g/l)</td>
<td>0.11</td>
<td>0.56</td>
<td>0.9-2.4</td>
<td>0.43</td>
<td>0.4-1.2</td>
</tr>
<tr>
<td>IgM (g/l)</td>
<td>6.11</td>
<td>0.56</td>
<td>0.4-1.2</td>
<td>0.4-1.2</td>
<td></td>
</tr>
</tbody>
</table>

Artemis - structure

T-B-SCID "atypical" T-B-SCID

partial deficiency of Artemis and...
EBV associated lymphoma

CD20

Ki67 (proliferative marker)

(lymph node biopsy)

Immunohistological features of lymphomas in P68 and P69

<table>
<thead>
<tr>
<th>Sites of involvement (H)</th>
<th>Immunohistochemistry</th>
<th>EBV markers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD20</td>
<td>CD3</td>
</tr>
<tr>
<td>P68 Cervical lymph node</td>
<td>Large cells</td>
<td>Rare small cells</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>P68 Striated muscle</td>
<td>Rare large cells</td>
<td>Large cells</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

(a) Lymphoproliferation was characterized as large B cells associated with small lymphocytes in both patients: clonal IgH proliferation without evidence of c-myc rearrangement.

Cytogenetic analysis in PHA activated T cells

- P68 Trisomy Chr. 9 (in lymphoma cells)
- P69 Translocation Chr. 7:14
- P70 nd
- P72 Inversion Chr. 7

Ig and TCR genes

hypomorphic mutations in Artemis

- partially preserved in vivo V(D)J activity
- polyclonal T and B cell populations, albeit reduced in number
- chromosomal instability
- aggressive EBV-associated B cell-lymphoma
  - clonality of B-cell proliferation
  - clonal chromosomal alteration in P68
  - general genomic instability

Artemis

a new « caretaker »

with tumor supressor role

immunodeficiency lymphoma

defects in NHEJ-factors
Jean-Pierre de Villartay
- Barbara Corneo
- Régina de Chasseval
INSERM U429
- Nicole Brousse
- Isabelle Callebaut
- Danielle Canioni
- Elizabeth Macintyre
- Christophe Pannetier
- Serge Romana

Alain Fischer
- Françoise Le Deist / C. Picard
- Marina Cavazzana-Calvo
- Jean-Laurent Casanova

and also...

atypical patients with hypomorphic mutations in Artemis
- several patients in the Paris cohort
- (S)CID phénotype with severe lymphopenia
- with defect in T and B cell function
- recurrent infections, ENT, bronchopulm
- chronic diarrhea
- note one patient with pulm. Asp niger
- EBV is a concern...
- MUD in one almost 6 y
- UCB 9.5/10 in a 27 mo

Chronic Inflammatory Bowel Disease as Key Manifestation of Atypical ARTEMIS Deficiency.

Chronic Inflammatory Bowel Disease as Key Manifestation of Atypical ARTEMIS Deficiency.
- recurrent diarrhea from the age of 9 months
- dx juvenile Crohn’s disease (biopsies chronically active inflammation with superficial fissuring ulceration)
- immune suppressive therapy (steroids, azathioprin, sulfasalazin, tacrolimus…)
- pneumonia, labial abscess
- chronic lymphopenia: PID considered at age of 6 years…
Chronic Inflammatory Bowel Disease as Key Manifestation of Atypical ARTEMIS Deficiency.

- 761 l/h/µl 63% yB TCR+/CD3+
- 146 CD3+ polyclonal repertoire
- 56 CD4+ only 2.5% naive cells
- 15 CD8+ proliferation N mitogens
- 40 B
- 530 NK
- normal Ig G A M, positive serological response

Chronic Inflammatory Bowel Disease as Key Manifestation of Atypical ARTEMIS Deficiency.

- novel homozygous point mutation Ex 6+1 g>a
- c461+1 g>a, splice donosite gt -> at
- several alternative transcripts, no normal transcripts
- residual V(D)J activity of some of these transcripts
- HYPOMORPHIC MUTATION

- haplo HSCT (father) follow up 3 years: alive and well

atypical manifestation?

think about a “leaky (S)CID” …
SCID T-B-NK+

- Rag1 ≈ 40%
- Rag2 ≈ 20%
- Artemis ≈ 40%