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V(D)J recombination and its defects

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Outline

- V(D)J recombination and its key players
- V(D)J recombination defects in SCID
- Case report of SCT of Artemis-deficient SCID patient
V(D)J recombination of the *IGH* gene

**DH to JH rearrangement**

**VH to DH-JH rearrangement**

**transcription**

**RNA splicing**

**translation**

**mature *IGH* mRNA**

**precursor *IGH* mRNA**

**signal joint**

**BREC**

**CD79a**

**CD79b**
**V(D)J recombination of the TCRB gene**

Vβ1, Vβ2, Vβ3, Vβ4, Vβ5, Vβn, Dβ1, Jβ1, Cβ1, Dβ2, Jβ2, Cβ2

**D → J rearrangement**

**V → D-J rearrangement**

**coding joints**

**transcription**

**translation**

*Erasmus MC*
B-lymphocyte

T-lymphocyte

T-lymphocyte
Identification and isolation of B-cell subsets

CD34+ lin− (defined as: CD3/13/19/33/56−)

UCB

CD34+ pro-B

CD34+ CD19+ CD19+ CD19+ CD19+ CD19+ CD19+

CD34− CD19+ CD10+ CD10+ CD10+ CD10hi

CD34− CD19+ CD10+ CD10hi

**IGH gene rearrangements quantified in the B-cell subsets**

*IGH gene complex (14q32.3)*

- **VH** (n=66)
- **DH** (n=27)
- **JH** (n=6)
- **C\textsubscript{\mu}

**Figure:**

- CD34\textsuperscript{+}lin\textsuperscript{−}
- pro-B
- pre-B-I
- pre-B-II large
- pre-B-II small
- immature-B
- mature-B\textsubscript{SmIg\textsuperscript{−}}
- mature-B\textsubscript{SmIg\textsuperscript{+}}
Summary of the Ig gene rearrangement processes during precursor-B-cell differentiation

UCB

CD34^{+}lin^{-} → pro-B → pre-B-I → pre-B-II large → pre-B-II small → immature B

bone marrow

VH-DJH in-frame selection

V_{\kappa}-J_{\kappa} / V_{\lambda}-J_{\lambda} in-frame selection

DH-JH

VH-DJH rearrangement

Kde (V_{\kappa}-Kde and intronRSS-Kde)

V_{\kappa}-J_{\kappa} rearrangement

V_{\lambda}-J_{\lambda} rearrangement

tonsil

mature-B Smlg_{\kappa}^{+}

mature-B Smlg_{\lambda}^{+}

Gene expression profiling of human CD34+lin– and precursor B-cell subsets

Human B-cell Differentiation

UCB → pro-B → pre-B-I → pre-B-II large → pre-B-II small → immature B → tonsil

- CD34^+ lin^-
- RAG1
- RAG2
- KLF2
- KLF4
- COPEB
- KLF12
- ERG
- ETS2
- ELK3
- ELF1
- ETS1
- SPIB
- OCT1
- OCAB
- OCT2
- POU4F1
- SMARCA5
- HIVEP3

- mature-B SmIg^κ^+
- mature-B SmIg^λ^+
EBF, LIG4 and RAG1 gene expression in human CD34+lin⁻ and precursor B-cell subsets

V(D)J recombination in detail

RAG1/RAG2 complex binds to RSS → DNA cleavage

1

hairpin coding ends

DNA-PKcs, Ku70/Ku80 and Artemis → hairpin cleavage

2

opened hairpins

TDdT activity

coding joint

ligation by DNA ligase IV, XRCC4 and XLF

blunt signal ends

signal joint
- Initiation by RAG1 and RAG2 is lymphoid specific

- Hairpin opening, end processing and ligation by non homologous end joining (NHEJ) pathway for DNA ds break repair (Ku70, Ku80, DNA-PKcs, LIG4, XRCC4, XLF-Cernunnos)
Outline

- V(D)J recombination and its key players
- V(D)J recombination defects in SCID
- Case report of SCT of Artemis-deficient SCID patient
Severe combined immunodeficiency (SCID)

- Clinical symptoms: opportunistic infections, protracted diarrhea, failure to thrive, presenting in the first months of life

- Many causative genetic defects have been described

- Immunological classification helpful to search for genetic defect
T-B-NK+ SCID is caused by defect in V(D)J recombination
T-B-NK⁺ SCID

- Approximately 20% to 30% of all SCID patients

- One third of these patients have mutations in the recombination activating genes (*RAG1* or *RAG2*)

- RAG proteins are essential for induction of V(D)J recombination of Ig and TCR genes
Flowcytometric analysis of precursor B-cell compartment in bone marrow

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tr>
<td>Pro-B</td>
<td>Pre-B-I</td>
<td>Pre-B-II</td>
<td>Immature B</td>
<td>Mature B</td>
<td></td>
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<tr>
<td>TdT</td>
<td>TdT</td>
<td>TdT</td>
<td>ψ</td>
<td>Igl</td>
<td>ψ</td>
<td>Igl</td>
<td>large</td>
<td>small</td>
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</table>

| CD22 | CD22 | CD22 | CD22 | CD22 | CD22 | CD22 | CD22 |
| CyCD79a | CyCD79a | CyCD79a | CyCD79a | CyCD79a | CyCD79a | CyCD79a | CyCD79a |
| CD19 | CD19 | CD19 | CD19 | CD19 | CD19 | CD19 | CD19 |
| CD34 | CD34 | CD34 | CD34 | CD34 | CD34 | CD34 | CD34 |
| CD10 | CD10 | CD10 | CD10 | CD10 | CD10 | CD10 | CD10 |
| CD20 | CD20 | CD20 | CD20 | CD20 | CD20 | CD20 | CD20 |

- **Pre-B-I**: TdT, ψ, Igl
- **Pre-B-II**: ψ, Igl
- **Immature B**: ψ, Igl, large
- **Mature B**: ψ, Igl, small
Composition of precursor B cell compartment in healthy children

average <5y

average 5-10y

average 10-18y

pro-B | pre-B-I | pre-B-II | immature B

0% 20% 40% 60% 80% 100%
CyIgμ/SmlgM/CD19 on bone marrow of RAG− SCID

purified CD19^ lymphogate healthy donor 1y7m

RAG-SCID2.2 0y1m

RAG-SCID3 0y4m

RAG-SCID4 0y2m
Precursor B-cell compartment in RAG deficient SCID patients

Average <5y (n=9)

RAG-SCID11

RAG-SCID9

RAG-SCID6-0y7m

RAG-SCID4-0y2m

RAG-SCID3-0y4m

RAG-SCID2.2-0y1m

RAG-SCID2.1-0y8m

pro-B

pre-B-I

pre-B-II

immature B
T- B- NK+ SCID patients without RAG gene mutations

- A number of these patients are sensitive to ionizing radiation

- Causative defect in DNA double strand break (dsb) repair via non-homologous end joining (NHEJ)
V(D)J recombination in detail

1. The RAG1/RAG2 complex binds to RSS and catalyzes DNA cleavage.

2. DNA-PKcs, Ku70/Ku80 and Artemis open the hairpins and catalyze hairpin cleavage.

3. TdT activity generates coding ends with sticky 3'-hydroxyl groups.

4. The coding ends are then ligated by DNA ligase IV, XRCC4, and XLF to form coding joint.

5. The signal ends are ligated by DNA ligase IV, XRCC4, and XLF to form signal joint.

6. Blunt ends are produced through the action of Blunt end repair enzymes.
Clonogenic survival assay of fibroblasts after ionizing radiation (Artemis 1,2,3)

% survival vs. Dose of X-rays (Gy)

- FN1 (wild type)
- NBS-1LBI
- Artemis-1
- Artemis-2
- Artemis-3.1
- Artemis-3.2
Principle of the V(D)J recombination assay

- Transfection of V(D)J recombination substrate into fibroblasts together with RAG1/RAG2
  - Wild type fibroblasts
  - Artemis-deficient fibroblasts
  - Artemis-deficient fibroblasts with wt Artemis or Artemis mutant
Signal and coding joint formation in Artemis− patients

Signal joint assay
- pGG49
- NV09F, NV08F
- FM30, DG147

Coding joint assay
- pGG51
- NV09F, NV08F
- FM30, DG147
Signal and coding joint formation in Artemis− patients

Signal joint assay:
- pGG49
- NV08F NV08F
- FM30 DG147

Coding joint assay:
- pGG51
- NV09F NV08F
- FM30 DG147

Cotransfection of RAG1, RAG2 and constructs with (+) or without (-) Artemis:

- signal joints: sj
  - -Art
  - +Art

- coding joints: cj
  - -Art
  - +Art

- FN1 control
- Artemis-1 (deletion exon 10-12)
- Artemis-2 (exon 5 G47T)
Composition of the precursor B-cell compartment in different types of T-B-NK+ SCID

- healthy children (n=6)
- RAG-SCID (n=7)
- Artemis-SCID (n=4)
Composition of DH-JH junctional region in B–/T– SCID patients

1. Assignment of D and J gene segments usage
2. Frequency of palindromic (P) nucleotides (caused by asymmetric “hairpin” opening)
3. Number of deleted nucleotides
4. Random insertion of nucleotides
## Composition of junction regions

<table>
<thead>
<tr>
<th></th>
<th>DH 3’ deletions</th>
<th>P</th>
<th>N</th>
<th>P</th>
<th>JH 5’ deletion</th>
<th>Total number of deletions</th>
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</thead>
<tbody>
<tr>
<td>Healthy controls</td>
<td>3.1</td>
<td>0.2</td>
<td>5.7</td>
<td>0.3</td>
<td>6.3</td>
<td>9.4</td>
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<tr>
<td>RAG-SCID</td>
<td>4.0</td>
<td>0.2</td>
<td>7.7</td>
<td>0.1</td>
<td>8.1</td>
<td>12.1</td>
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<tr>
<td>Artemis-2</td>
<td>3'del</td>
<td>DH</td>
<td>DH</td>
<td>P-nucleotides*</td>
<td>N-nucleotides</td>
<td>P-nucleotides</td>
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<tr>
<td>DH1-26</td>
<td>0</td>
<td>GTAGT</td>
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<tr>
<td>DH1-26</td>
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<td>GTAGTA</td>
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<tr>
<td>DH2-15</td>
<td>0</td>
<td>GGAGTAGT</td>
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<td>TCTCAAAGTGTAACAAA</td>
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<td>DH2-21</td>
<td>0</td>
<td>GGAAT</td>
<td></td>
<td></td>
<td>TTTAGACCAACAAA</td>
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<td>DH3-22</td>
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<td>GAGT</td>
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<td>DH3-9</td>
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<td>DH3-22</td>
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<td>DH4-23</td>
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<td>DH4-23</td>
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<td>CGAGTAATAGGGGC</td>
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<td>DH4-23</td>
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<td>GG</td>
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<tr>
<td>DH5-12</td>
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<td>CCA</td>
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<td>CGTAAACC</td>
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<td>CTCC</td>
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<td>-4</td>
<td>CTT</td>
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<tr>
<td>DH5-12</td>
<td>-1</td>
<td>TTTAAGTAAT</td>
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</tbody>
</table>

* Underlined nucleotides can be derived from both D and J side.*
## Composition of DH-JH coding joints

<table>
<thead>
<tr>
<th></th>
<th>DH 3' del</th>
<th>P</th>
<th>N</th>
<th>P</th>
<th>JH 5' del</th>
<th>Total P</th>
<th>Total del</th>
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</thead>
<tbody>
<tr>
<td>Healthy controls (15)</td>
<td>-4.2</td>
<td>0.1</td>
<td>7.9</td>
<td>0.1</td>
<td>-6.0</td>
<td>0.2</td>
<td>10.2</td>
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<tr>
<td>RAG-SCID (15)</td>
<td>-4.0</td>
<td>0.2</td>
<td>7.7</td>
<td>0.1</td>
<td>-8.1</td>
<td>0.2</td>
<td>12.1</td>
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<tr>
<td>Artemis-SCID (53)</td>
<td>-1.9</td>
<td>3.0</td>
<td>4.0</td>
<td>3.8</td>
<td>-1.1</td>
<td>6.7</td>
<td>3.3</td>
</tr>
</tbody>
</table>
Case report of T-B-NK+ SCID

- Girl from consanguineous parents
- In first year of life no problems
- In second year development of infections of respiratory tracts and candidiasis
- Successively, development of chronic diarrhea and failure to thrive.
- At 18 months suspicion for immunodeficiency due to hypogammaglobulinemia
- Improvement with immunoglobulin substitution and broad-spectrum antibiotics.
- BMT was initiated, but patient died during conditioning period
Low numbers of T and B cells found in peripheral blood

<table>
<thead>
<tr>
<th></th>
<th>Patient</th>
<th>Mean RAG-SCID</th>
<th>Mean Artemis-SCID</th>
<th>Control (x10^9/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B cells</td>
<td>0.01</td>
<td>0.001</td>
<td>0.001</td>
<td>0.2-1.6</td>
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<tr>
<td>T cells</td>
<td>0.23</td>
<td>0.2</td>
<td>0.5</td>
<td>0.7-4.2</td>
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<tr>
<td>NK cells</td>
<td>0.5</td>
<td>1.1</td>
<td>1.1</td>
<td>0.09-0.9</td>
</tr>
</tbody>
</table>
Clonogenic survival assay of fibroblasts after ionizing radiation

Dose of X-rays (Gy)

% Survival

FN1 (wild type)

Artemis

Patient SC2
Composition of the precursor B-cell compartment in different types of T-B-NK+ SCID

- Patient SC2
- Artemis-SCID (n=4)
- RAG-SCID (n=7)
- Healthy children (n=6)

Legend:
- pro-B
- pre-B-I
- pre-B-II
- immature B
### Composition of coding joints of patient SC2

<table>
<thead>
<tr>
<th>$D_h$ segment</th>
<th>3$'$ deletion</th>
<th>N nucleotides</th>
<th>5$'$ deletion</th>
<th>$J_h$ segment</th>
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<tr>
<td>$D_h$2–21</td>
<td>0</td>
<td>G</td>
<td>-7</td>
<td>$J_h$4</td>
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<tr>
<td>$D_h$5–24</td>
<td>-13</td>
<td>-</td>
<td>-20</td>
<td>$J_h$6</td>
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<td>$D_h$2–2</td>
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<td>G</td>
<td>-20</td>
<td>$J_h$4</td>
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<td>$D_h$2–15</td>
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<td>CTGT</td>
<td>-15</td>
<td>$J_h$5</td>
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<tr>
<td>$D_h$3–9</td>
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<td>C</td>
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<td>$J_h$2</td>
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<td>$D_h$2–15</td>
<td>-14</td>
<td>CG</td>
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<td>$J_h$4</td>
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<tr>
<td>$D_h$4–11</td>
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<td>-</td>
<td>-19</td>
<td>$J_h$4$^A$</td>
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<td>$D_h$4–17</td>
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<td>GCCT</td>
<td>-14</td>
<td>$J_h$5</td>
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<tr>
<td>$D_h$4–23</td>
<td>-12</td>
<td>-</td>
<td>-29</td>
<td>$J_h$4$^{A,B}$</td>
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<tr>
<td>$D_h$5–12</td>
<td>-2</td>
<td>GGG</td>
<td>-9</td>
<td>$J_h$2</td>
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<td>$D_h$5–24</td>
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<td>GGA</td>
<td>-32</td>
<td>$J_h$4$^B$</td>
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<td>$D_h$2–24</td>
<td>-9</td>
<td>TCGGACAC</td>
<td>-19</td>
<td>$J_h$4</td>
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<tr>
<td>$D_h$5–12</td>
<td>-2</td>
<td>CGGTGACA</td>
<td>-14</td>
<td>$J_h$4</td>
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<tr>
<td><strong>Average</strong></td>
<td>-12.2 ± 15.9</td>
<td>2.8</td>
<td>-16.0 ± 8.5</td>
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</tr>
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</table>
### Composition of DH-JH coding joints

<table>
<thead>
<tr>
<th></th>
<th>DH 3’ del</th>
<th>P</th>
<th>N</th>
<th>P</th>
<th>JH 5’ del</th>
<th>Total P</th>
<th>Total del</th>
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<td>7.9</td>
<td>0.1</td>
<td>-6.0</td>
<td>0.2</td>
<td>10.2</td>
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<td>RAG-SCID (15)</td>
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<td>7.7</td>
<td>0.1</td>
<td>-8.1</td>
<td>0.2</td>
<td>12.1</td>
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<td>Artemis-SCID (53)</td>
<td>-1.9</td>
<td>3.0</td>
<td>4.0</td>
<td>3.8</td>
<td>-1.1</td>
<td>6.7</td>
<td>3.3</td>
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<td>SC2</td>
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<td>2.8</td>
<td>0</td>
<td>-16.0</td>
<td>0.1</td>
<td>28.2</td>
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RS-SCID caused by a deletion of three nucleotides in DNA ligase IV

- First patient with RS-SCID due to DNA Ligase IV mutation

- Different from described Ligase IV syndrome patients, because no microcephaly, growth delay
- A Ligase IV mutation can give rise to RS-SCID
- Clinical spectrum of Ligase IV mutations is broadened

<table>
<thead>
<tr>
<th></th>
<th>Immunodeficiency</th>
<th>Microcephaly and growth delay</th>
<th>Radiosensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS-SCID</td>
<td>++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>LIG4 syndrome</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Leukemia</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

- Can other NHEJ defects be expected?
  Yes → Cernunnos
Conclusions

- V(D)J recombination defects result in absence of T and B cells (SCID or growth retardation, microcephaly, and immunodeficiency)

- Characteristic block in precursor-B cell differentiation

- V(D)J defects can be caused by mutations in RAG1 or RAG2

- If not, fibroblasts are tested for radiosensitivity → if RS, than a defect is expected in one of the components of NHEJ

- Analysis of DH-JH coding joints gives insight in which step of V(D)J recombination is defective (Artemis-deficiency: long P – LIG4-deficiency: large deletions)

- Fibroblasts can also be used for in vitro V(D)J recombination studies or complementation
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Albert Pastink

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Tuba Turul
Ilhan Tezcan

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Ewa Bernatowska

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Outline

- V(D)J recombination and its key players
- V(D)J recombination defects in SCID
- Case report of SCT of Artemis-deficient SCID patient
Differences in outcome of SCT of B-negative SCID and B-positive SCID

- HLA non-identical T-cell depleted SCT are significantly better for B-positive SCID than for B-negative SCID (J.Pediatr 1999;134:740-8)

- Reduced survival of B-negative SCID associated with:
  - diminished rate in engraftment
  - higher frequency of chronic GVHD
  - slower recovery and lower rate of T/B function
Artemis-deficient SCID

- Female patient diagnosed with SCID at age of 5 months (failure to thrive, pneumonia due to Pneumocystis carinii and CMV infections)

- Agammaglobulinemia, no B-cells, T-cells were of maternal origin

- At age of 7 months, SCT from HLA-identical brother, uneventful post-transplantation course

- Gradual rise in T-cells of donor origin, T-cells of mother disappeared within one year

- However, no B-cell engraftment and patient remained dependent on immunoglobulin substitution
“Lack of Space hypothesis”

No B-cell engraftment post-SCT in B-negative SCID patients because of lack of physical space in bone marrow due to the presence of a relatively high frequency of early precursor B-cells (pro-B and pre-B-I), which are not eradicated with mild pre-SCT conditioning.

Van der Burg et al. Hematol 2006; 91:1705-9
Analysis of precursor B-cell compartment at diagnosis and post-SCT

Van der Burg et al. Hematol 2006; 91:1705-9
Short tandem repeat (STR) analysis

patient

donor
Post-SCT all pre-B-I cells of patient

patient

donor

Pre-B-I

Pre-B-II large

Pre-B-II small

Immature

Average <5y (n=9)

Diagnosis

Post-BMT1

pro-B (CD22+CD19+)
pro-B-I (CD34+CD10+CD20-)
pro-B-II large (CD34-CD10+CD20-)
pro-B-II small (CD34-CD10+CD20+)
immature B (CD34-CD10+CD20++)
Second SCT

- Artemis-deficient SCID patient received a second SCT from same donor

- Pre-SCT conditioning with busulphan
B-cell recovery after 2nd SCT

Average <5y (n=9)

Diagnosis

Post-BMT1

Post-BMT2

- pro-B (CD22+CD19-)
- pre-B-I (CD34+CD10+CD20-)
- pre-B-II large (CD34-CD10+CD20-)
- pre-B-II small (CD34-CD10+CD20+)
- immature B (CD34-CD10+CD20++)
Post-SCT2 precursor B-cells of donor origin

Patient

Donor

Pre-B-I

Pre-B-II large

Pre-B-II small

Immature

Average <5y (n=9)

Diagnosis

Post-BMT1

Post-BMT2

Van der Burg et al. Hematol 2006; 91:1705-9
Conclusion

B-cell recovery after stem cell transplantation of Artemis-deficient SCID requires elimination of autologous bone marrow precursor B-cells
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