Engrafted Maternal T Cells in a Severe Combined Immunodeficiency Patient Express T-Cell Receptor Variable Beta Segments Characterized by a Restricted V-D-J Junctional Diversity

By Alessandra Sottini, Eugenia Quiròs-Roldan, Luigi D. Notarangelo, Alberto Malagoli, Daniele Primi, and Luisa Imberti

To better understand the peculiar functional behavior of engrafted maternal T cells in a severe combined immunodeficiency (SCID) patient, we characterized, at the molecular level, the T-cell repertoire of a SCID child with a high number of engrafted, mature, activated lymphocytes. We found that, although these transplacentally acquired T cells express a random set of T-cell receptor variable beta (TCRβ) segments, the TCRβ transcripts are characterized by an extremely restricted V-D-J junctional diversity. Only a few TCRβ clones were dominant among the TCRBV4, TCRBV6, and TCRBV20 populations in engrafted cells, whereas the same TCRβ chains expressed by the mother’s lymphocytes had the expected junctional heterogeneity. Highly diverse and polyclonal junctions were also expressed by maternal cells activated in mixed lymphocyte reaction by Epstein-Barr virus (EBV)-transformed B lymphocytes from the patient, indicating that the strong clonal selection that characterizes the engrafted cells repertoire is probably not due to allorecognition. Furthermore, we report that the repertoire of the transplacentally acquired lymphocytes is dynamic over time and is characterized by waves of expression and contraction of selected clones, expressing different TCRβ segments. These results help to explain some of the abnormal functional behaviors of engrafted maternal cells and raise new questions regarding the mechanisms responsible for the restricted clonal diversity.

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ALTHOUGH THE PHENOMENON of maternal lymphocytes engraftment in severe combined immunodeficiency (SCID) patients has been known for several years, its biologic and clinical implications are still largely unresolved. Maternal lymphocytes may be present in variable amounts in immunodeficient children and may persist for months in their blood circulation. Engraftment of maternal T cells in an HLA-mismatched setting should lead to immune activation. However, while engrafted T cells usually bear surface activation markers, they appear anergic, as shown by failure of in vitro proliferation to mitogens, as well as an inability to protect the host against infections. In addition, the clinical consequences of maternal T-cell engraftment in an immunodeficient host are variable and often mild, if any, graft-versus-host disease (GVHD) is observed in an environment that should be an ideal target for alloergic T-cell activity.

This unusual functional behavior of maternal T cells in SCID patients has been explained by Knobloch et al. as the result of the profoundly reduced T-cell receptor (TCR) diversity. Using a panel of monoclonal antibodies (MoAb) against T-cell receptor variable beta (TCRβ) segments (the new World Health Organization-International Union of Immunological Societies recommended nomenclature is used throughout this manuscript), these investigators demonstrated the lack of one or several TCRβ segments in all the SCID patients analyzed and a dramatic expansion of other TCRβ chains in half of the children. Consequently, the functional alteration of these cells has been ascribed to an insufficient TCR diversity. This hypothesis, however, fails to explain the lack of response of engrafted lymphocytes to T-cell ligands, such mitogens, and anti-CD3 MoAb that act through nonclonally distributed receptors. Thus, several aspects concerning the functional behavior and the TCR repertoire of transplacentally acquired T lymphocytes in SCID patients deserve further studies.

Here, we report the molecular analysis of the TCRβ repertoire of engrafted maternal T cells in a SCID child. This patient had a remarkably high number of engrafted mature, activated T cells expressing TCRβ segments are characterized by an extremely restricted V-D-J junctional diversity. Furthermore, our data suggest that the repertoire of the engrafted cells is very dynamic over time and is characterized by waves of expression and contraction of particular clones, possibly in response to environmental antigens or superantigens. These results help to explain some of the abnormal functional behaviors of these cells and raise new questions regarding the mechanisms responsible for such a restricted clonal diversity.
in maternal T and B cells, whereas a random pattern of X-chromosome inactivation of SCID was based on nonrandom X-chromosome inactivation of SCID included evaluation of the adenosine deaminase (ADA) differently methylated on the active versus the inactive X-chromosome. ADA activity was measured from EBV-transformed patient's cells. On day 6, the lymphocytes were phenotypically characterized and a pure preparation of CD45+ cells was obtained.

Table 1. Phenotype of the SCID Patient's Lymphocytes

<table>
<thead>
<tr>
<th>MoAbs</th>
<th>Patient</th>
<th>Mother</th>
<th>Infant Controls (3 to 6 mo old)*</th>
<th>Adult Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD1</td>
<td>0</td>
<td>1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>CD2</td>
<td>85</td>
<td>92</td>
<td>65 ± 13</td>
<td>78.5 ± 6.5</td>
</tr>
<tr>
<td>CD3</td>
<td>53</td>
<td>87</td>
<td>60 ± 15</td>
<td>74 ± 7</td>
</tr>
<tr>
<td>CD4</td>
<td>52</td>
<td>60</td>
<td>43 ± 17</td>
<td>45 ± 7</td>
</tr>
<tr>
<td>CD45RA</td>
<td>1</td>
<td>15</td>
<td>35 ± 12</td>
<td>ND</td>
</tr>
<tr>
<td>CD45RO</td>
<td>52</td>
<td>ND</td>
<td>8 ± 6</td>
<td>29 ± 8</td>
</tr>
<tr>
<td>CD8</td>
<td>1.7</td>
<td>15</td>
<td>21 ± 12</td>
<td>30 ± 10</td>
</tr>
<tr>
<td>CD45RA</td>
<td>0.7</td>
<td>18</td>
<td>15 ± 5</td>
<td>26.5 ± 5</td>
</tr>
<tr>
<td>CD45RO</td>
<td>1.2</td>
<td>ND</td>
<td>5 ± 2</td>
<td>18 ± 9</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>76</td>
<td>6.5</td>
<td>20 ± 9</td>
<td>10 ± 6</td>
</tr>
<tr>
<td>CD19</td>
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<td>29</td>
<td>6.8</td>
<td>17 ± 10</td>
<td>7.5 ± 2.5</td>
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<td>CD25</td>
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<td>6 ± 2</td>
<td>5 ± 2.5</td>
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<tr>
<td>CD16</td>
<td>1</td>
<td>10</td>
<td>10 ± 6</td>
<td>13 ± 7</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.

*The data represent the mean values ± SD obtained with lymphocytes from 10-1 to 6 month-old children.
†The data represent the mean values ± SD obtained with lymphocytes from 20 adult healthy individuals.

Chimerism analysis for detection of maternal lymphocytes was done by HLA-typing using standard, complement-mediated cytotoxicity assay and by sequence-specific oligonucleotide primer hybridization (SSOPH). Following bone marrow transplantation, full chimerism, with the presence of the donor's peripheral blood mononuclear cells (PBMC) and no evidence of the patient's or mother's PBMC was demonstrated 12 days after transplantation, and this situation remained unchanged over time.

Lympohocyte preparation and cell cultures. Blood samples were obtained on two separate occasions before bone marrow transplantation was performed. In both cases, DNA analysis with the highly polymorphic marker D1S80 showed mixed chimerism in PBMC from the patient. PBMC were prepared from the SCID patient and his mother by Ficoll Hypaque gradient centrifugation and immediately used. Control PBMC were isolated from healthy individuals and age-matched children. B lymphocytes were prepared by negative selection from PBMC after neuraminidase-treated sheep erythrocytes. Epstein-Barr virus (EBV)-transformed cells, obtained by culturing B cells with the B95/8 cell line supernatant, were grown in RPMI 1640, 20% fetal calf serum (GIBCO Laboratories, Grand Island, NY), CD4+, CD25 +, and CD19 + cells were prepared from the patient's mother lymphocytes by using magnetic microspherical beads coated with specific MoAbs (Dynabeads, Dynal, Oslo, Norway), following manufacturer's instructions. The purity of these preparations, measured by cytofluorimetry, was always 98%. In vitro cell cultures were performed in complete RPMI 10% (vol/vol) heat-inactivated human AB-serum pooled from prescreened donors, in the presence or absence of the following stimulators, immobilized for 2 hours on the plastic surface: anti-CD3 MoAb (100 ng/mL; Ortho Diagnostic System, Raritan, NJ), human recombinant interferon-gamma (100 U/mL, Hoffman La Roche, Basel, Switzerland), phytohemagglutinin (PHA-P, 10 μg/mL, Wellcome Diagnostic, Daeestock, UK), or the staphylococcal enterotoxin A (SEA), B (SEB), C1 (SEC1), C2 (SEC2), C3 (SEC3), D (SED), and E (SEI) (100 ng/mL, Serva, Feinbiochemica, Heidelberg, Germany). Mixed lymphocyte reaction (MLR) was set up by culturing for 6 days 1 × 10^6 responder mother's lymphocytes and stimulator cells obtained from EBV-transformed patient's cells. On day 6, the lymphocytes were phenotypically characterized and a pure preparation of CD45+ cells was obtained.

Phenotypic analysis. The following mouse MoAbs were used: fluorescein isothiocyanate conjugated (FITC) anti-CD1, -CD2, -CD3, -CD4, -CD8, HLA-DR, -CD19, -CD20 (Ortho)-CD16, and -CD25 (Becton Dickinson, Mountain View, CA) and phycoerythrin (PE) conjugated anti-CD45RA (Coulter, Hialeah, FL), and CD45RO (Becton Dickinson). Immunofluorescence analysis was performed by using a flow cytometer (FACScan, Becton Dickinson, Eremborgem-aalst, Belgium) equipped with an argon-ion laser. Cells were
Table 3. Functional Capacities of Patient Engrafted Maternal T Cells

<table>
<thead>
<tr>
<th>Stimulation</th>
<th>Patient</th>
<th>Mother</th>
<th>Infant Controls (3 to 6 mo old)</th>
<th>Adult Controls†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Background</td>
<td>3,704</td>
<td>2,452</td>
<td>3,294 ± 2,443</td>
<td>2,597 ± 3,065</td>
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<tr>
<td>PHA</td>
<td>6,510</td>
<td>84,365</td>
<td>65,324 ± 29,483</td>
<td>76,226 ± 27,387</td>
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<tr>
<td>Anti-CD3 MoAb (100 µg/mL)</td>
<td>5,721</td>
<td>66,653</td>
<td>45,646 ± 29,023</td>
<td>42,064 ± 15,437</td>
</tr>
<tr>
<td>Anti-CD3 hrlL-2 (100 U/mL)</td>
<td>10,800</td>
<td>76,490</td>
<td>59,404 ± 30,493</td>
<td>ND</td>
</tr>
<tr>
<td>Anti-CD3 hrlL-2 (1,000 U/mL)</td>
<td>7,211</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>hrL-2 (100 U/mL)</td>
<td>6,806</td>
<td>2,496</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>hrL-2 (1,000 U/mL)</td>
<td>7,511</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>SEA</td>
<td>23,558</td>
<td>54,022</td>
<td>40,909 ± 29,509</td>
<td>38,640 ± 10,654</td>
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<tr>
<td>SEB</td>
<td>21,812</td>
<td>58,148</td>
<td>41,993 ± 30,878</td>
<td>33,757 ± 22,971</td>
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<tr>
<td>SEC1</td>
<td>19,948</td>
<td>56,319</td>
<td>33,896 ± 17,349</td>
<td>41,147 ± 16,838</td>
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<tr>
<td>SEC2</td>
<td>18,925</td>
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<td>21,143 ± 9,169</td>
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<tr>
<td>SEC3</td>
<td>45,329</td>
<td>60,012</td>
<td>32,570 ± 14,650</td>
<td>61,452 ± 24,205</td>
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<tr>
<td>SED</td>
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<td>43,772</td>
<td>25,546 ± 7,948</td>
<td>41,107 ± 24,205</td>
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<tr>
<td>SEE</td>
<td>17,488</td>
<td>65,063</td>
<td>32,437 ± 16,584</td>
<td>34,242 ± 16,048</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.

*The data represent the mean values ± SD obtained with lymphocytes from ten 3- to 6-month-old children.
†The data represent the mean values ± SD obtained with lymphocytes from 20 adult healthy individuals.

Cloning of PCR products. The PCR products were purified by cutting the band with the expected size from New Sive low melting agarose 2.5% gel and eluting the melted gel through ion-exchange resin column (Qiagen tip 5; Qiagen Inc, Chatsworth, CA). Purified DNA fragments were ligated to pCR II vector. Plasmids were grown in 2'VaF' Escherichti coli cells in Luria Bertani agar plates and single plaques were picked up and expanded according to the manufacturer’s instructions (TA cloning kit; Invitrogen Corp, San Diego, CA).

Hybridization of cDNA libraries. Recombinant plaques were transferred from LB agar plates to Hybond-N+ membranes as described, and the membranes were first hybridized with a TCRBV probe (5' GTC GCT GTG TTT GAGCCA TCA GAA 3') specific probe and then with TCRBV4, TCRBV6, TCRBV20, or TCRBV22-specific probes using DIG Oligonucleotide 3'-End labeling kit and DIG Nucleic Acid Detection kit (Boehringer Mannheim, Mannheim, Germany).

DiA sequencing reaction. TCRBV4, TCRBV6, TCRBV20, and TCRBV22 positive plaques were selected, and plasmid DNA was purified and sequenced by the dideoxy chain-termination technique using Sequenase version 2.0 DNA Sequencing Kit (US Biochemical Corp, Cleveland, OH). Sequences were compared with published data relative to TCRBV, BD, BJ, and BC segments.

RESULTS

Demonstration of maternal T cells in peripheral blood of the SCID patient. The patient was referred to the Paediatric Division for suspected immunodeficiency. The phenotypic analysis (Table 1) showed that the majority of his T lymphocytes were CD2+CD3+CD1− mature cells. Virtually all of these lymphocytes were CD4+CD5+CD1− cells and coexpressed HLA-DR and CD25 molecules, suggesting a generalized state of activation of the T-cell population. The number of B lymphocytes was at the upper limit, while CD16+ cells were virtually absent. This phenotypic profile remained constant for several months.

HLA class II typing was performed by cytotoxicity on patient’s and mother’s B cells, while HLA class I typing...
T cells could selectively respond to superantigens was unexpected because these cells have been reported to possess a limited T-cell repertoire diversity. It was important, therefore, to assess whether alterations in TCRBV usage could also be detected in the engrafted cells of our patient.

The analysis of the TCRBV usage on engrafted maternal lymphocytes was performed by PCR, using a degenerated primer that amplifies B chain rearrangements containing virtually all of the known TCRBV regions. As a control, we used cDNA derived from CD4 T maternal lymphocytes obtained by magnetic beads separation. The relative expression of each TCRBV segment was determined by a colorimetric method as previously described. Figure 1 shows that the general pattern of TCRBV usage of the engrafted T cells did not deviate significantly from that of maternal CD4 T lymphocytes. In both T-cell populations, there was no evidence of a restricted utilization of TCRBV segments; there were, however, some differences in the relative expression of particular TCRBV segments in the two samples. The engrafted T cells, in fact, were characterized by an apparent enrichment of TCRBV4 and TCRBV20 segments, as well as a reduced expression of TCRBV1, TCRBV3, and TCRBV6 chains. To verify this result, we amplified the above-mentioned TCRBV chains with TCRBV family-specific primers and we showed the specificity of the amplified products by using, as probe, a common bioin-conjugate TCRBC oligonucleotide. With this strategy we fully confirmed the results obtained with the degenerated PCR strategy.

**Oligoclonality of TCRBV segments in the SCID patient.** While the apparent lack of TCRBV restriction detected in T cells from the SCID patient explains their capacity to proliferate to various exogenous superantigens, it does not explain their impaired response to conventional ligands (Table 3). Therefore, the size of the actual TCR repertoire of these cells was investigated in more detail. Because TCR diversity is highly skewed towards the V-D-J junctional regions, we determined, in a first series of experiments, the nucleotidic sequences of several TCRBV20 transcripts derived from the engrafted and the maternal CD4 + T cells. The data in Fig 2 show that the pool of TCRBV20 sequences, derived from the engrafted T-cell population, were characterized by a surprisingly low heterogeneity of junctional diversity that defined only three different TCRBV20 T-cell clones. Clones MC4 and MC5 were identical to the dominant clone, expressing TCRBJ2S1, except for a Gly-Glu replacement in the CDR3 of one of their TCRBV20 transcripts.

![Fig 1. TCRBV expression of PBMC from (II) engrafted maternal lymphocytes and (III) maternal CD4+ cells. Data are expressed as the percentage of the signal obtained with the individual TCRBV specific probes.](image-url)
TCR JUNCTICIAL DIVERSITY IN SCID PATIENT

Two dominant clones, that differed for only two amino acids, expressed the TCRBJ156 segment, one of the least used TCRBJ segments in TCRBV transcripts (Rosenberg et al. and Quirós-Roldan et al., submitted for publication). As in the case of TCRBV20 clones, the TCRBV4 (Fig 3) and TCRBV6 (Fig 4) groups of sequences derived from the mother’s CD4+ cells were highly heterogeneous and, most importantly, none of the dominant transcripts detected in the child were found to be expressed by maternal T cells.

TCR oligoclonality is not determined by antihost reactivity. To investigate whether the restricted TCR diversity is biased against the host histocompatibility antigens, we analyzed the TCRBV usage of maternal cells activated in MLR against irradiated EBV-transformed B cells prepared from the host. Cells were phenotypically characterized after 6 days of culture. Cytofluorimetric analysis, performed on activated cells, showed that 38% of T lymphocytes were CD4+CD45RO+ and 38% were CD8+CD45RO+ (data not shown). Cells cultured in MRL were used to isolate a pure preparation of responding activated maternal lymphocytes from which total RNA was isolated and utilized for TCR repertoire study. The analysis of TCRBV usage of these cells was not very informative, except for the fact that there was no evidence of an enrichment of TCRBV20 sequences (data not shown).

To address whether the repertoire of engrafted cells may result from clonotype selection in response to host MHC, we sequenced several TCRBV20 and TCRBV4 transcripts obtained from these MLR-activated lymphocytes. Figure 5 shows that both sets of sequences were characterized by extensive junctional diversity, and there was no evidence of selection of particular clones. Furthermore, none of these sequences were similar to the corresponding TCRBV transcripts that were found to be selectively enriched in the engrafted T cells. Taken together, these data suggest that the clonotypic selection detected in maternal T cells in the SCID patient does not reflect a selective expansion of clones against MHC antigens of the host. Therefore, it is likely that different and perhaps more complex mechanisms, possibly involving the interplay between Ig and TCR regions, may influence the distribution of the TCR repertoire in these patients.

Modulation of the engrafted T cells repertoire with time. To determine whether the repertoire of engrafted T cells was stable or changed over time, the analysis of the T-cell repertoire was repeated after 6 months. At this time, the cell phenotype deviated from the profile observed at the time of the first diagnosis, because an increase of CD19+ B lymphocytes and a marked reduction of CD3+CD4+ were observed. The proportion of CD8+ cells was also increased, probably due to the expansion of a natural killer (NK) cell, because most of CD8+ cells coexpressed CD57, but not CD3. Because NK cells do not bear TCR molecules, the analysis of the TCRBV usage even at this time reflects the repertoire expressed by CD3+CD4- lymphocytes. The result of PCR amplification demonstrated that a strong modulation of the TCR repertoire had occurred during the 6 months period; the decrease of the highly expressed TCRBV chains was

![Fig 3. Junctional TCRBV4 (BV4) sequences obtained from engrafted maternal lymphocytes (left) and maternal CD4+ cells (right). Amino acid sequences were deduced from nucleotide sequences and displayed as a standard one-letter code. Only the last 10 amino acids of the TCRBV4 chain and the first 3' amino acids of TCRBJ (BJ) segments are shown. The putative CDR3 regions, defined according to Chothia et al., are underlined.](image-url)
counterbalanced by an increase of new segments, such as TCRBV1351, TCRBV17, and TCRBV22 (Fig 6). These differences do not reflect experimental artifacts because the analysis of TCR repertoire, carried out by using family-specific primers and by showing the specificity of the amplified products with the colorimetric method, gave similar results. To determine whether these differences reflected the normalization of TCRBV diversity or, alternatively, the occurrence of selection events acting on specific clones, we sequenced several TCRBV22 cDNA clones. We choose to sequence TCRBV22 segments because this chain is usually expressed at a low level in lymphocytes of normal individuals. Figure 7 shows that the sequences of these highly modulated segments were again characterized by an oligoclonal pattern of junctional regions.

Taken together, these results demonstrate that the TCR repertoire of engrafted T cells is highly dynamic, but remains restricted in terms of junctional diversity, even for a long period of time.

**DISCUSSION**

In this report, we analyzed, at the molecular level, the TCR repertoire of transplacentally-acquired maternal T cells in a SCID child. Our patient, sent to the Paediatric Unit with a suspected immunodeficiency, had a remarkably high number of engrafted mature activated T cells, all expressing

<table>
<thead>
<tr>
<th>Class</th>
<th>BV4</th>
<th>BM</th>
<th>BW</th>
<th>BJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0818</td>
<td>DHSLYCSE</td>
<td>IN</td>
<td>IN</td>
<td>IN</td>
</tr>
<tr>
<td>M0819</td>
<td>DHSLYCSE</td>
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<td>IN</td>
<td>IN</td>
</tr>
</tbody>
</table>

**Fig 5.** Junctional TCRBV4 (BV4) (left) and TCRBV20 (BV20) sequences (right), obtained from maternal lymphocytes activated in MLR by EBV-transformed cells prepared from the patient. Amino acid sequences were deduced from nucleotide sequences and displayed as a standard one-letter code. Only the last 10 amino acids of the TCRBV chain and the first 3' amino acids of TCRBJ (BJ) segments are shown. The putative CDR3 regions, defined according to Chothia et al., are underlined.

**Fig 4.** Junctional TCRBV6 (BV6) sequences obtained from engrafted maternal lymphocytes (left) and maternal CD4+ cells (right). Amino acid sequences were deduced from nucleotide sequences and displayed as a standard one-letter code. Only the last 10 amino acids of the TCRBV6 chain and the first 3' amino acids of TCRBJ (BJ) segments are shown. The putative CDR3 regions, defined according to Chothia et al., are underlined.
the CD4+CD45RO+CD45RA+ phenotype, that coexisted with B cells belonging to the patient. These T lymphocytes were found to be apparently unresponsive to PHA and to anti-CD3 MoAb, even in conjunction with IL-2, but proliferated well to exogenous superantigens. The proliferative response to superantigens was unexpected. Given the described restriction of TCRBV expression of maternal T cells in SCID mice and humans, we were surprised to find that engrafted cells responded to superantigens stimulation. The existence of profound alterations of the TCR repertoire, affecting specific TCRBV chains, should, in fact, theoretically result in a skewed pattern of proliferation in response to different superantigens, unless other signalling pathways (i.e., via MHC class II molecules) are used.

The analysis of the TCR repertoire, performed by PCR on T cells obtained from our patient, showed a nonrestricted pattern of TCRBV expression compared with that of maternal T cells. However, we observed significant differences in the two repertoires with respect to the amount of some TCRBV transcripts. TCRBV3 and TCRBV6 appeared to be more expressed in the mother, while TCRBV4 and TCRBV20 segments were preferentially expressed in the child. The high expression of TCRBV20 was of particular interest, because this segment is usually poorly represented in normal subjects. The sequence data of TCRBV4, TCRBV6, and TCRBV20 segments demonstrated a dramatic expansion of a restricted set of clones in the engrafted cells, while the transcripts of the mother’s T cells were characterized by the expected junctional heterogeneity. The possibility that the restricted usage of only a few identical sequences in the clones prepared from the patient was due to contaminations can be ruled out by the fact that for the entire procedure (PCR, cloning, and sequencing) the mother’s and the child’s lymphocytes were run in parallel. The implication of this result is that the TCR repertoire of the engrafted cells is not restricted in terms of TCRBV genes usage, but that each TCRBV segment utilizes only minimal junctional diversity. The restricted usage of functional diversity remained constant over time, although there was a clear modulation of the expression of the different TCRBV segments. This finding implies that the restriction is not reversible and that the few clones available in the SCID patient expand or contract, presumably in response to environmental antigens or superantigens.

The most immediate explanation for this finding is that the narrow repertoire is biased against host histocompatibility antigens. Our results, however, argue against this possibility. First, we found that maternal T cells triggered with EBV-transformed B cells retained their functional activity on a second stimulation. Second, none of the dominant TCRBV20 and TCRBV4 transcripts, found in the engrafted cells, were detected in the MRL-activated lymphocytes. Third, the expressed repertoire of TCRBV chains changed considerably over time. An alternative explanation, already proposed by Knobloch et al., is that the restricted repertoire may be due to the negative selection of T cells having specificity for host histocompatibility antigens. However, this hypothesis is not compatible with the extreme restricted diversity of the TCRBV clones observed in our experiments.

Alterations of the expression of TCRBV chains are often suggestive of an involvement of superantigens in the selection of specific TCRBV specificities. Superantigens differ from conventional antigens because they selectively interact with MHC class II and the TCRBV polypeptide chains of the TCR. Consequently, superantigen triggered cells are characterized by the expression of particular TCRBV chains with highly heterogeneous junctional diversity. The redundancy of the CDR3 regions detected in the TCRBV sequences analyzed in the present study, excludes the possibility that superantigen molecules participate in the shaping of the repertoire of engrafted cells.

One intriguing possibility is that, under normal conditions, the expression of TCRBV22 is often suggestive of an involvement of superantigens in the selection of specific TCRBV specificities. Superantigens differ from conventional antigens because they selectively interact with MHC class II and the TCRBV polypeptide chains of the TCR. Consequently, superantigen triggered cells are characterized by the expression of particular TCRBV chains with highly heterogeneous junctional diversity. The redundancy of the CDR3 regions detected in the TCRBV sequences analyzed in the present study, excludes the possibility that superantigen molecules participate in the shaping of the repertoire of engrafted cells.

The TCRBV expression of PBMC from engrafted maternal lymphocytes, obtained 6 months after the first examination. Data are expressed as the percentage of the signal obtained with the individual TCRBV specific probes.

![Fig 6. TCRBV expression of PBMC from engrafted maternal lymphocytes, obtained 6 months after the first examination. Data are expressed as the percentage of the signal obtained with the individual TCRBV specific probes.](image)

The restricted repertoire may be due to the negative selection of T cells having specificity for host histocompatibility antigens. However, this hypothesis is not compatible with the extreme restricted diversity of the TCRBV clones observed in our experiments.

<table>
<thead>
<tr>
<th>Clones</th>
<th>TCRBV</th>
<th>D3N</th>
<th>BJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMYAA6</td>
<td>SAMYFCASS</td>
<td>GGTRI</td>
<td>SPLHFGNGTRTVL 1.6</td>
</tr>
<tr>
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<td>SAMYFCASS</td>
<td>TPTGGRV</td>
<td>YNEQFGPGTRGLTVL 2.1</td>
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Fig 7. Junctional TCRBV22 sequences obtained from engrafted maternal lymphocytes. Amino acid sequences were deduced from nucleotide sequences and displayed as a standard one-letter code. Only the last 10 amino acids of the TCRBV22 chain and the first 3' amino acids of TCRBJ (BJ) segments are shown. The putative CDR3 regions, defined according to Chothia et al., are underlined.
nonspecific factors eliminate the totality of maternal T cells in the host, while in SCID patients the compromised immune system allows some of these cells to escape negative selection and to expand in the child. Although we cannot definitively exclude the occurrence of this event, we found it unlikely because one of the dominant TCRBV clones used the TCRBJ1S6 segment. It is now well established that human TCR sequences use a clear pattern of preference of TCRBJ usage, with TCRBJ2S7, TCRBJ1S1, and TCRBJ2S1 used more frequently and TCRBJ1S6, TCRBJ1S3, and TCRBJ1S4 rarely used (Rosenberg et al 1991 and Quiros-Roldan et al, submitted for publication). It is likely, therefore, that the cells with the highest probability to escape a nonspecific mechanism of negative selection are those that are preferentially represented in the original population. Because this prediction does not match with our findings, we favor the hypothesis that the restricted TCR repertoire of engrafted cells reflects a positive selection pressure that promotes the expansion of few clones from an initial pool of unselected lymphocytes.

Little is known about the diversity of the B-cell repertoire in SCID patients, but Ig diversity may be limited. Circulating serum Ig have been shown to regulate the magnitude and quality of the immune response, to participate in the selection of emergent bone marrow repertoires, and to be of therapeutic value in a number of autoimmune diseases. These observations are consistent with the notion that the B-cell repertoire plays an important regulatory role in the immune system, although the precise mechanisms that mediate these effects have not been elucidated. At large, several observations indicate that the immune system or, at least, its compartments producing antibodies embody a network with many other self-components including TCR variable gene products. It is, perhaps, not surprising that mature T cells that are transferred in an environment containing a limited Ig diversity do not find the necessary stimuli to fully expand their receptor diversity.

Although the mechanisms responsible for the selection of an highly restricted T-cell repertoire in engrafted cells remain speculative, the central finding has strong implications for understanding the functional impairment of these cells. It is tempting to speculate that the oligoclonality associated with the expression of each different TCRBV segment may fully explain the functional data. The expression of a complete TCRBV repertoire would, in fact, provide the necessary substrates for superantigen activity, while the limited junctional diversity will fall short of providing the necessary diversity for full responsiveness to foreign antigens.

In conclusion, our data define a yet undescribed restricted TCR repertoire in engrafted cells, whereby a complete expression of TCRBV elements is associated with an extremely limited level of junctional diversity. The insufficient TCR diversity, however, does not fully explain the functional impairment of these cells. It is likely that in SCID patients the combined action of independent mechanisms presides, on one side, the selection of a narrow repertoire and, on the other side, the modulation of the triggering potential of transplacentally-acquired maternal T cells.

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Engrafted maternal T cells in a severe combined immunodeficiency patient express T-cell receptor variable beta segments characterized by a restricted V-D-J junctional diversity

A Sottini, E Quiros-Roldan, LD Notarangelo, A Malagoli, D Primi and L Imberti