Severe Combined Immunodeficiency (SCID) in Man: B Cell-negative (B-) SCID Patients Exhibit an Irregular Recombination Pattern at the JH Locus

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Summary

Human severe combined immunodeficiency (SCID) patients were analyzed by a polymerase chain reaction assay for their recombination capability at the DQ~J. region of the immunoglobulin heavy chain locus. Five patients with B cells (B+ SCID) exhibited a recombination pattern also observed in healthy persons. In contrast, six patients lacking B cells (B- SCID) showed a grossly altered rearrangement pattern characterized by the (partial) absence of regular DQ~J, recombinations and the presence of abnormal rearrangements. These events were caused by deletions surpassing the boundaries of immunoglobulin coding elements and thus resemble the pattern of deletional recombinations previously described in SCID mice.

Human SCID is a rare, heterogeneous congenital disorder of the lymphatic system (1, 2). Affected infants are characterized by severe and persistent infections due to the impaired function of B and T lymphocytes. Without bone marrow transplantation the patients usually die before the age of two years (1).

In approximately 15–20% of patients, SCID is associated with a deficiency of the enzyme adenosine deaminase (ADA)1. Additional immunodeficiencies are characterized by abnormalities of the enzymes purine nucleoside phosphorylase (PNP) and ecto-5'-nucleotidase (3).

Rare cases of SCID can also result from the inappropriate expression of restriction elements of the HLA loci (4-6), defective IL-2 production (7, 8), the failure of T cells to respond to IL-1 (9), impairments in the T cell receptor/CD3 complex (10, 11), abnormalities of the cytokkeleton and from disturbances of signal transduction pathways within T cells (12). Frequently, human SCID is inherited as a X-linked, recessive trait (McKusick no. 30040 SCID X1), characterized by the absence of mature T cells and normal or elevated numbers of B cells (13). The SCID subentities mentioned above all bear detectable numbers of B cells in their peripheral blood. However, occasionally patients have been reported in which B and pre-B cells are completely absent (B- SCID), suggesting a distinct type of SCID with a profound defect of the lymphocyte development (14).

One of the crucial steps for the development of functional lymphocytes is the correct joining of distinct subgenic elements (V(D)J recombination) to generate coding sequences for Ig or TCR variable regions (15, 16). The joining process is mediated by signal sequences (RSS: recombination signal sequences) flanking each coding element. The juxtaposition of two coding elements is usually associated with modifications at the junctional region, due to the loss of a few bases at the coding ends and de novo insertion of nucleotides (N-nucleotides), most likely mediated by the enzyme terminal desoxynucleotidyl transferase (TdT) (17). The process of N-region insertion is developmentally regulated in B and T cells, with the number of N-nucleotides included in the junctional region increasing with time after birth (18–20). Alternatively, extra bases may represent inverted repeats (palindromes) at the termini from the adjoining coding segments (P-nucleotides) (18, 19).

The rearrangement of the V, (D) and J elements is tightly controlled, occurs in a preferential order (e.g., D,D,J recombinations, subsequently V,J recombinations) and is lineage specific (TCR loci are usually not completely rearranged in B cells and vice versa) (21).

The rearrangement process is thought to be mediated by an enzymatic machinery (V(D)J "recombinase") which is common to B and T cells (22).

A mouse model in which the correct assembly of the subgenic V, (D), J elements is defective, has been described (23).

Abbreviations used in this paper: ADA, adenosin deaminase; DTH, delayed type of hypersensitivity; PNP, purine nucleotide phosphorylase; RSS, recombination signal sequence; TdT, terminal desoxynucleotidyl transferase.

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This autosomal recessive SCID mutation (24) adversely affects early lymphoid differentiation. Thus young mice, homozygous for SCID, lack functional T and B lymphocytes and phenotypically resemble B- SCID patients. However, transformed cell lines which have a phenotype characteristic of immature lymphocytes can be obtained from SCID mice. The crucial finding made in those transformed lymphocytes was that rearrangements (on the IgH and TCRB loci) often deleted the entire J cluster and extended into the flanking regions (25-29). Recent analysis of the TCRy (30) and Igk loci (31) confirm these findings. These studies support that the recombinase system in SCID mice is capable of cleaving the DNA at the correct position (between RSS and coding elements) and joining the signal elements, but fails to mediate the formation of normal coding joints (31, 32). The crucial finding made in those transformed lymphocytes was that rearrangements (on the IgH and TCRB loci) often deleted the entire J cluster and extended into the flanking regions (25-29). Recent analysis of the TCRy (30) and Igk loci (31) confirm these findings. These studies support that the recombinase system in SCID mice is capable of cleaving the DNA at the correct position (between RSS and coding elements) and joining the signal elements, but fails to mediate the formation of normal coding joints (31, 32). These studies support that the recombinase system in SCID mice is capable of cleaving the DNA at the correct position (between RSS and coding elements) and joining the signal elements, but fails to mediate the formation of normal coding joints (31, 32). It remains to be established whether correct rearrangement events observed in older SCID mice are due to spontaneous revertants or rather reflect a low-frequency "leakiness" of the mutated recombinase (33) and whether the murine SCID mutation is caused by a more general defect in DNA repair (34).

In this report, we have examined the rearrangement capability of B cell negative (B-) and B cell positive (B+) human SCID patients. Since in contrast to the murine SCID model, the establishment of transformed cell lines poses a major problem, we used a sensitive PCR assay to characterize endogenous DQβ2 to Jβ rearrangements. We conclude from these experiments that the B- SCID patients are characterized by an impaired rearrangement process at the Jβ region analogous to the defect in SCID mice.

### Table 1. Clinical and Immunological Characterization of Severe Combined Immunodeficiency (SCID) Patients

| Phenotype* | Sex | Age | CD20 | CD3 | TCRα/β | CD15 | CD16 | Maternal T cells$ |
|------------|-----|-----|------|-----|--------|------|------|------------------|
| B+ SCID patients | D. B. | m | 8 mo | 18 | 14 | 14 | 25 | 39 | + |
| | D. D. | m | 1 d | 65 | 7 | ND | ND | 7 | ND |
| | K. K. | m | 17 mo | 20 | 0.2 | ND | 31 | 1 | ND |
| | F. P. | m | 1 d | 27 | 5 | ND | 54 | 2 | - |
| | M. Z. | m | 4 mo | 26 | 1 | ND | ND | 0 | - |
| B- SCID patients | V. M. | f | 1 d | 0 | 45 | 38 | 36 | 12 | + |
| | T. C. | m | 2 mo | 0 | 0 | 0 | 34 | 14 | - |
| | P. P. | f | 1 mo | 0 | 67 | 73 | 5 | 26 | + |
| | J. B. | m | 1 mo | 0 | 44 | 48 | 32 | 17 | ND |
| | J. J. | m | 3 mo | <1 | 70 | 64 | 8 | 19 | + |
| | S. K. | m | 6 mo | 0 | 3 | 2 | 3 | 67 | + |

* Percentage of PBMC which were stained by indirect immunofluorescence.
1 The age of the patients at diagnosis.
$ Maternal T cells are present (+) or absent (-) in an in vitro culture system of PBMC (35).
Polymerase Chain Reaction. PCR was essentially performed as suggested by Saiki et al. (38). A 100 µl reaction mixture contained 0.5–1 µg of genomic DNA (when digested with BamH I, Bgl II, or EcoRI I), 50 pmol of each 5’ and 3’ oligonucleotide primer, 200 µmol/l dATP, dCTP, dGTP, and dTTP, 10 mmol/l Tris-HCl pH 8.3, 50 mmol/l KCl, 2.5 mmol/l MgCl₂, and 0.001% (w/v) gelatin. The reaction mixture was exposed for 10 min to 302 nm UV light on a Vilber Lourmal supplilitor (Bachhofer, Reutlingen, Germany) before the amplification of genomic DNA. Oligonucleotide primers are listed below (see: Oligonucleotides) and their position within the J₅ region is depicted in Fig. 1. After an initial denaturing step of 10 min at 95°C, 1.5 U Ampli TA polymerase (Cetus Corp., Norwalk, CT) and 1 µl of gp32 (Pharmacia) (39) were added and 30 amplification cycles were run in an automatic PCR processor (Bio-Med, Theres, Germany). Following the initial denaturation, the melting, annealing and elongation steps were performed at 94°C for 2 min, 56°C for 90 s and 72°C for 5 min, respectively. The final cycle was followed by an additional extension for 10 min at 72°C.

10% of the amplification products were separated on an agarose gel (0.8% or 1.2%) and studied by Southern blot analysis.

Isolation, Cloning and Sequencing of Amplified DNA. 20–50% of the amplified DNA was cleaved with appropriate restriction enzymes (Pharmacia) to allow for directional cloning, ligated into pBSKSM13+ (Stratagene, Heidelberg, Germany) or pT7T3U18 (Pharmacia) vectors and transformed into R.R.lacZAM15 Escherichia coli.

White colonies were picked and rescreened by colony hybridization with oligonucleotides 3, 4, or 5 (see below).

Plasmid DNA for sequence analysis was isolated from each recombinant clone via alkaline lysis. Sequencing followed the dideoxy chain termination method described by Sanger (40) using T7 DNA polymerase according to the instructions of the manufacturers (US Biochemical, Cleveland, Ohio) (Pharmacia).

Evaluation of the sequences was done with version 6.2 of the University of Wisconsin Genetics Computer Group (GCG) sequence analysis package (41).

Probes. For hybridization studies we used two probes, each covering part of the J₅-J₆ locus. Probe a (Fig. 1) is a 2.5 kb fragment starting 5’ of J₅ and ending at the Bgl II site of the Cµ enhancer (42). A second probe (probe b, Fig. 1) was generated by PCR amplification of undigested genomic DNA using oligonucleotides 2 and 6. A plasmid containing the germline configuration of the D₅s2-J₅ region was obtained after cloning and colony hybridization. A BamH I/Nco I digest yielded a 1.1 kb fragment which spans the region from oligonucleotide 2 to base 1103. Nucleotide numbering follows a published sequence of the J₅ locus (43).

Oligonucleotides. Synthetic oligonucleotides were prepared according to published sequences (43). The oligonucleotides were used for hybridizations (H), PCR (P), or sequence analyses (S).

Oligonucleotide 1: (P), 5’ ACC CAG CAC TGG TGG ACA C; Oligonucleotide 2: (P), an additional BamH I site (underlined) for cloning was synthesized. 5’ GCC GAA GCC TCG AGT GGC ACG AGT GTC AAC; Oligonucleotide 3: (H, S), 5’ CCT ACC AGC CGC AGG GT; Oligonucleotide 4: (H), 5’ GGC CTA CAA AAA CCA TGC TCC; Oligonucleotide 5: (H, S), 5’ CGT GTG CCC TTT CCC CCA GAC; Oligonucleotide 6: (P), an additional Sal I site is underlined. 5’ GCC GTC GAC GCC AGT AGC AGA AAA CAA AGG.

In addition, we used T₅ and T₇ oligonucleotides (Pharmacia) for sequence analyses.

Results

D₅s2-J₅ Polymerase Chain Reaction Assay. We decided to make use of the enormous sensitivity of the PCR technique for the detection of D₅s2-J₅ associated rearrangements. The assay was designed such that one primer pair could amplify the germline band and all six possible D₅s2-J₅ rearrangements simultaneously. D₅s2-J₅ rearrangements were visualized by Southern blot analysis.

We initially amplified the bone marrow DNA of healthy donors. Fig. 2 A is the result of one representative experiment. Amplification products of expected size were visible and represent a “ladder” of D₅s2-J₅ rearrangements. Reproducible amplifications especially of the larger products were only achieved after inclusion of gp 32 in our PCR approach (39).
Figure 2. Southern blot analyses of PCR amplification products representing D.52-J.1 rearrangements. The DNA was separated on a 0.8% agarose gel and hybridized to probe a (Fig. 1 A). As size marker, Hind III digested λ DNA was included. (A) Genomic DNA of normal bone marrow was either used without restriction enzyme digestion (lane 1 and 4) or with prior cleavage by EcoN I (lane 2 and 5) or BamH I (lane 3 and 6); BamH I does not cut within the DHQ52-J.1 region. The DNA was amplified with oligonucleotides 1 and 6 (lane 1–3) or with primers 2 and 6 (lane 4–6). (B) Genomic DNA digested with EcoN I was amplified with oligonucleotide 2 (Fig. 1) was used as upstream primer instead of oligonucleotide 1. In addition, hybridization with specific oligonucleotides representing J.2-J.6 sequences confirmed the conclusion that respective bands represent rearrangement events. Analysis of the amplification products which have been digested with Nco I (cuts between J.2 and J.3), ApaL I (cuts between J.3 and J.4), or Hinc II (cuts between J.4 and J.5) supported this view (data not shown).

The specificity of the assay was proven by the absence of amplification products when nonhuman DNA (salmon sperm or mouse liver DNA) (Fig. 2 B) was used. In addition, a panel of human cell lines (Fig. 2 B) was analyzed to test the fidelity of the reaction. In fact, only the germline signals were observed upon amplification of DNA obtained from all six cell lines tested.

Sequence Analysis of D.45-J.1 Associated Rearrangements of Healthy Persons. Amplification products of bone marrow DNA (cut with EcoN I) of two healthy donors were cloned. After selection by lacZ α complementation, rearrangement events were scored by hybridization to oligonucleotide 3 and/or 5 (Fig. 1). Clones hybridizing to oligonucleotide 4 were excluded from sequence analyses since they represented most likely germline amplifications. We sequenced 29 clones (clone 1–29, Fig. 3 A) which hybridized to oligonucleotide 3 and/or 5, and one clone (clone 30, Fig. 3 A) which hybridized only to oligonucleotide 5, representing two D.45-J.4, five D.45-J.5, and 23 D.45-J.6 rearrangements. The distribution of J.6 rearrangements probably reflects a double bias in amplification and cloning efficiency where fragments of smaller size are favoured. The J.4 and J.6 elements that were sequenced were in complete accordance with those published by Yamada et al. (44) and showed some nucleotide substitution compared with another report (43). The J.6 sequences derived from our analysis rather represent a composition of J.6 nucleotide sequences recently published (43, 44). Six of 30 clones (20%) lacked evidence for N nucleotides insertion (clone 1, 3, 8–11). The GC content of N regions was 65.4%. Both values correspond to published data (45). Twenty of 30 clones (66.7%) lacked evidence for N nucleotides insertion (clone 1, 3, 8–11). The GC content of N regions was 65.4%. Both values correspond to published data (45). Twenty of 30 clones (66.7%)
used the last two 3' nucleotides before the 3' RSS of Dq52 as junctional nucleotide. The junctional nucleotides at the 5' site of JH6 were spread more evenly, reaching from the first to the seventeenth base with a peak at base nine.

Possible pallindromic sequences (P elements) (18) were detected in 47% of the clones which had not deleted nucleotides at the ends of the coding elements (clone 14, 18, 19, 27, 28, 30). We obtained one clone (clone 30, Fig. 3 A) which had vector sequences directly followed by P/N nucleotides and the JH6 element. The presence of the vector sequence most likely was due to a 3 nucleotide P element insertion at the 3' site of Dq52 which creates a BamHI site and juxtaposes P/N elements to vector sequences.

Of six clones without N nucleotides (Fig. 3 A), three contained overlapping sequences of Dq52 and JH6 (clone 1, 8, 9). The overlap of one clone (clone 1) (Fig. 3 B) encompassed seven nucleotides. Clone 8 and 9 (Fig. 3 A) overlapped by one base. One possible mechanistic explanation for such an overlap (as seen in clone 1) could be a homologous recombination event, as previously suggested (20).

**Figure 4.** Analysis of Dq52-JH rearrangements in PBMC DNA from B- SCID patients. Oligonucleotides 2 and 6 were used for amplification, probe a for hybridization. Hind III digested DNA served as size marker. As template the following (DNA) samples (cut with EcoN I) were included: Lane 1, water, treated identically like the samples; lane 2, PBMC (normal); lane 3, patient 1 (D. B.); lane 4, patient 2 (D. D.); lane 5, patient 3 (K. K.); lane 6, patient 4 (F. P.); lane 7, patient 5 (M. Z.).
nation “ladder”. However, the amplification products represent a heterogenous pattern: one patient (patient 9, Table 1) did not show any rearranged fragment (Fig. 5, lane 7), all other patients revealed the absence of some bands (Fig. 5, lane 4–9) and, in addition, several patients (patients 6, 10, and 11) showed amplification products which were of exceptional size (Fig. 5, lane 4–9), i.e., not representing regular D₉ₛ₂-J₉ recombinations.

The latter interpretation was supported by the hybridization of the filter used in Fig. 5 A to probe b (Fig. 5 B). Patients 6 and 10, who had irregularly hybridizing bands with probe a, lost some of those signals after hybridization to probe b. Thus the upstream regions of these putative D₉ₛ₂-J₉ rearrangements have obviously lost hybridizable sequences which span from oligonucleotide 2 to sequences between J₉₂ and J₉₅. Amplification of PBMC DNA of patient 6 and 10 showed similar patterns of D₉ₛ₂-J₉ recombinations as observed in bone marrow DNA (data not shown). Genotypic analysis of the immunoglobulin gene status was done on PBMC or bone marrow DNA after digestion with Bgl II or Hind III and Southern blot analyses using probe a (Fig. 1). The probe hybridized in all patients to 3.9 kb Bgl II and to 9.5 kb Hind III germline fragments, indicating that no gross DNA abnormalities were present within the analyzed Ig gene loci (data not shown).

Sequence Analysis of B⁻ Severe Combined Immunodeficiency (SCID) Patients. Nucleotide sequences derived from amplification products of bone marrow DNA (EcoN I or Bgl II digests) of two B⁻ SCID patients were in marked contrast to the results obtained in healthy donors. We detected only two clones (S₆E₂ and S₁₀B₃) containing P nucleotide insertions, which resembled regular J₉₄ rearrangements (Fig. 6 A). However, the other clones showed deletions of different sizes (Fig. 6 B). In all clones the 5' breakpoint occurred between the upstream amplification primer and D₉ₛ₂. The 3′ breakpoint occurred always within a J₉ element; clone S₁₀B₂ exhibits a deletion of 51 nucleotides of J₉₅ sequences, thus by far exceeding the normal range of J₉₅ nucleotide loss in normal DJ junctions. Three of the deletion clones contained N sequences, supporting the view that this step in V(D)J recombinations is not mutated in B⁻ SCID patients.

Hybridization of a clonospecific sequence derived from one deletion clone (clone S₁₀B₁) (Fig. 6 B) to the Southern blot analysis of amplification products (Fig. 7 C) additionally supported the interpretation that aberrant fragments in the Southern blot analysis actually represent irregular rearrangements.

Abnormal Rearrangements In Normal Bone Marrow Donors. Since we detected abnormal recombinations in B⁻ SCID patients, we asked if recombination failures are unique to B⁻ SCID patients or if these irregular recombinations can also be detected in normal individuals. To enrich for the detection of deletional events, we digested the DNA of two normal bone marrow donors and three B⁻ SCID patients with Bgl II prior to the analyses; Bgl II cuts at position 331, thus between the 5′ amplification oligonucleotide and D₉ₛ₂. Therefore only those events that have deleted position 331 can serve as intact template during the amplification procedure besides a small amount of uncut regular D₉ₛ₂-J₉₅ rearrangement events. Fig. 7 A shows the result of a Southern blot analysis of amplification products after hybridization to probe a. In fact, healthy persons (Fig. 7, lane 1 and 2) as
weill as SCID patients (Fig. 5, lane 3–5) exhibit irregularly sized fragments. Upon rehybridization of the filter to probe b (Fig. 7 B), most of the irregular bands either lose their intensity or are no longer visible, indicating that deletions between oligonucleotide 2 and J.2-J.3 must have occurred. After cloning Bgl II digested amplified DNA of a healthy individual, one clone (clone 31) (Fig. 8) had an irregular recombin-ation pattern, since a deletion of 1584 bp (from base pair 273 to 2857) was detected. The point of recombination did not include any coding elements nor were any cryptic RSS visible in the vicinity of the breakpoints. Thus no principle difference between normal bone marrow donors and B- SCID patients is observed in this analysis. We conclude that irregular rearrangements are present, albeit rare in normal individuals.

Discussion

We have studied the pattern of Ig gene rearrangements in five B- and six B- human SCID patients by a PCR assay amplifying the Dm82-Jm region. Either by Southern blot or by sequence analyses the amplification products were characterized in more detail. At the Southern blot level B- SCID patients showed a normal Dm82-Jm recombination pattern, indicating a regular function of the “recombinase” machinery in this disease subentity.

In contrast, all six B- SCID patients showed a grossly disturbed recombination pattern in the Southern blot analysis, with the lack of or with faulty Dm82-Jm rearrangements. Sequence analyses of the amplification products of B- SCID patients showed that a high percentage of the rearrangements included deletions which surpass the boundaries of coding elements. This finding resembles the molecular defect in the SCID mouse (25–29). Since the used PCR approach allowed the detection of either normal rearrangements or of joinings with relatively small deletions on both sides flanking either Dm82 or the Jm cluster, we expected to amplify only a minor proportion of recombinations and especially deletion events. Given the recombination defect in B- SCID patients, this might explain why each patient exhibits a characteristic lack of usage of particular Jm. Additionally, the clonal expansion (possibly driven by antigenic stimuli) of a few cells bearing a normal Dm82-Jm rearrangement might explain the oligoclonal pattern of normal recombinations detected in B- SCID patients. Whether these bands are generated due to the leakiness of the recombination machinery remains to be established.

The enzymatic pathway of Ig gene recombination has been divided into specific steps which are either mediated by the RSS or by nonspecific DNA processing enzymes (46). After RSS are identified by the recombination protein(s) and (probably) brought into physical proximity, cutting occurs at the RSS borders. Upon cleavage, the recombinogenesis machinery might continue to hold the coding and signal ends in close proximity to one another. The cut intermediates have to be quite stable since they must be long-lived enough to allow polymerases and exnucleases to modify their termini. Thereafter, the ligation of the open ends will restore the integra-tion of the DNA strand. The two normal clones sequenced in our B- SCID patients and the presence of N nucleotides in the deletion clones indicate that the B- SCID recombi-nation machinery is competent for the recognition of RSS, cutting at the RSS sites and the addition of P and N nucleo-tides. The defect associated with B- SCID may rather repre-sent a step marking the end of the trimming process of the coding elements. Along this line, one of the two alleles of a transformed cell line derived from a B cell negative (0% B cells, 7% T cells) SCID patient showed a rearrangement of the Dm82 element to a region 60 nucleotides downstream from Jm, while the second allele represented a normal Dm82-Jm recombination (14).

Our data also provide evidence for the current view that irregular Ig recombinations are not exclusively observed in B- SCID patients. The existence of one sequenced deletion clone and several irregular fragments in the Southern blot analysis of healthy individuals points to this fact. Occasionally lymphocyte precursors escape the otherwise tightly controlled recombination process of normal lymphopoiesis. Like-
Figure 7. Analysis of D<sub>nal</sub>-J<sub>d</sub> associated rearrangements in bone marrow DNA obtained from healthy probands and B<sup>-</sup> SCID patients. Oligonucleotides 2 and 6 were used for amplification; probe a (A), probe b (B) and the clonospecific oligonucleotide 5' GGT GAA CCC CTT TGA CTA CTG GGC 3' (C) were hybridized to the identical filter. The separation was done on a 1.2% agarose gel. Markers as in Fig. 2. The following samples pretreated with Bgl II were included in the analyses: Lane 1, healthy individual 1; lane 2, healthy individual 2; lane 3, patient 6 (V. M.); lane 4, patient 7 (T. C.); lane 5, patient 10 (J. J.). Arrowheads in A indicate bands which show a reduced intensity after hybridization to probe b (B).

Figure 8. Sequence of a deletional clone obtained after amplification of Bgl II digested DNA of a healthy individnal. Sequence comparison was done according to published reports (43, 44). Arrowheads represent RSS.

One of the critical points concerning our PCR assay is the presence of maternal T cells in B<sup>-</sup> SCID patients since it has been realized that T cells can undergo DaJ<sub>d</sub> but no complete V<sub>d</sub>AJ<sub>d</sub> rearrangement (48). These maternal T cells could account for the presence of the normal rearrangements in our B<sup>-</sup> SCID panel. Two observations argue against this implication: (a) B<sup>-</sup> SCID patient 7 (T.C.) who had no maternal T cells on the basis of fluorescence analysis and in vitro culture of PBMC (Table 1) showed a similar recombination pattern as other B<sup>-</sup> SCID patients; (b) amplification of 99% pure (by cytofluorometry) peripheral T cells of two normal donors yielded the normal recombination ladder as observed with PBMC or bone marrow of healthy persons (K. Schwarz, unpublished data). In vitro culture of PBMC or of lymphocytes from different organ biopsies of patient 6 (V. M.) and patient 10 (J. J.) yielded cell lines with >90% CD3 positive cells which were of maternal origin only. Yet, preliminary PCR analyses of those CD3 positive cell lines revealed only the germline band (K. Schwarz, unpublished data). Thus those maternal cells either have no D<sub>nal</sub>-J<sub>d</sub> rearrangement at all or they use other D<sub>n</sub> sequences outside of the range of our PCR primers.

Whether pre T cells of the B<sup>-</sup> SCID patients recombine TCR genes with faults remains to be established. Patients lacking maternal T cells might allow to approach this problem. The identification of B<sup>-</sup> human SCID patients who may be characterized by a defect within the recombination machinery analogous to SCID mice opens new avenues as to the differential diagnosis of SCID patients and marks a step towards the molecular definition of these heterogeneous entities.
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