High Frequency of Normal DJn Joints in B Cell Progenitors in Severe Combined Immunodeficiency Mice

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Summary

The severe combined immunodeficiency (scid) mouse has a defective V(D)J recombinase activity that results in arrested lymphoid development at the pro-B cell stage in the B lineage. The defect is not absolute and scid mice do attempt gene rearrangement. Indeed, ~15% of all scid mice develop detectable levels of oligoclonal serum immunoglobulin and T cell activity. To gain more insight into the scid defect and its effect on V(D)J rearrangement, we analyzed DJn recombination in scid bone marrow. We determined that DJn structures are present in scid bone marrow and occur at a frequency only 10-100 times less than C.B-17+/+. The scid DJn repertoire is limited and resembles fetal liver DJn junctions, with few N insertions and predominant usage of reading frame 1. Moreover, 70% of the DJn structures were potentially productive, indicating that normal V(D)J recombinants should be arising continually.

The formation of functional Ig and TCR genes requires the rearrangement of several genetic elements encoding the variable regions: V, J, and, in some cases, D (1). The mechanism of this recombination is only now beginning to be elucidated, and most of what we know about the process is derived from studies of its substrates and its products (for review, see references 2 and 3). The process of rearrangement is mediated by an enzymatic system, the V(D)J recombinase, which is targeted by the recombination signal sequences (RSS) flanking the elements to be rearranged. RSS consist of three parts; a heptamer, a 12- or 23-bp spacer, and a nonamer. As a result of V(D)J recombination, two types of junctions are formed: signal joints and coding joints (4). In the signal joints, which are usually precise, RSS are joined in a head-to-head orientation. Coding joints, which are usually imprecise, have a few nucleotides added and/or deleted at the coding termini.

V(D)J recombination is essential for normal lymphoid development. Mice deficient for the recombinase activating genes, Rag-1 and Rag-2, as well as scid mice, have defective V(D)J recombinase activity (5-9), and lymphoid development in all these mice is arrested at the pro-B cell stage. Unlike the Rag-1⁻ and Rag-2⁻ mice, however, scid mice attempt gene rearrangement, but these attempts fail to produce functional receptors. In Abelson murine leukemia virus (A-MuLV)-transformed scid bone marrow lines (5, 9) and in long-term cultures of scid bone marrow (10), there are large deletions in the coding joints even though signal joints are relatively normal (8, 11).

The scid defect is leaky. Approximately 15% of all scid mice develop detectable levels of oligoclonal serum Ig and T cell activity (12-15). The incidence of leakiness varies with both the age of the mice and their environment (12). Petrini et al. (16) have postulated that a genetic reversion occurs in a B cell precursor permitting normal V(D)J recombination in subsequent daughter cells. In support of this hypothesis, some functional T cell clones derived from leaky scid mice have normal rearrangements on their nonexpressed TCR alleles. This result is unexpected from the high frequency of abnormal coding joints found in A-MuLV scid lines (16, 17). Further, normal TCR rearrangement at the δ DJ locus has been observed in scid thymocytes (18, 19). However, there is no evidence of attempted Vδ rearrangement or rearrangement at the β and γ loci (20). An alternative hypothesis to explain the leaky phenotype is that scid mice make productive rearrangements at a higher frequency than previously expected (see below), so that a few B cells are produced each day depending on the chance occurrence of several rearrangement events. Thus, whether or not a mouse becomes leaky depends in part on whether a chance clone of B cells is stimulated by antigen before it is eliminated as part of the normal B cell turn-over.

Abbreviations used in this paper: A-MuLV, Abelson murine leukemia virus; BM, bone marrow; RF, reading frame; RSS, recombination signal sequence.

1 Abbreviations used in this paper: A-MuLV, Abelson murine leukemia virus; BM, bone marrow; RF, reading frame; RSS, recombination signal sequence.
To gain more insight into scid defect and its effect on the mechanism of V(D)J rearrangement, we have analyzed DJ<sub>4</sub> recombination products in scid mice. Using a quantitative PCR assay devised to determine the extent of DJ<sub>4</sub> formation (21), we found DJ<sub>4</sub> structures in scid mice occur at a frequency only 10-100 times less than in C.B-17+/+ mice. Sequencing of these structures revealed that the scid DJ<sub>4</sub> repertoire is limited and resembles fetal liver DJ<sub>4</sub> junctions, with few N insertions and predominant usage of reading frame 1. Moreover, 70% of these structures were potentially productive, indicating that normal V(D)J recombinants should be arising continually in scid mice.

Materials and Methods

**Mice and Cell Lines.** The C.B-17<sup>scid</sup> (referred to in the text as scid mice) and C.B-17 wild type (referred to in the text as C.B-17+/+) were originally obtained from Melvin Bosma (Fox Chase Cancer Center, Philadelphia, PA). C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All mouse strains were bred and maintained in the animal colony of the Ontario Cancer Institute (Toronto, Canada) under defined flora conditions. Nonleaky and leaky scid mice were differentiated on the basis of serum Ig detected by Ouchterlony diffusion. Bone marrow (BM) and spleens were removed from individual mice at 6 or 27 wk of age. A-MuLV cell lines were derived and maintained as described (22).

**DNA Preparation.** Single-cell suspensions were prepared from the adult BM and spleens using standard procedures (23). Genomic DNA was isolated from the BM, spleen, and cultured cell lines by the "spooling" method as previously described (22).

**Oligonucleotide PCR Primers.** The DFS primer is a 31mer hybridizing to the 5' RSS of the D<sub>j</sub><sub>4</sub> elements of both the Dsp and the DFL families. Its sequence is 5'-AGGGATCCTTGTGAAGGGATCTTACTGTG-3'. The DQ52 primer is 5'-GCGGAGCACACAGTGCACGATGGAC-3'. It is a 26mer specific for DQ52; it hybridizes to the region within the 5' spacer through the heptamer and contains all of the DQ52 coding region. The J<sub>c</sub> primer is 5'-AAGGACTCTTGAGAGGCTTACCCG-3'. It is a 26mer containing sequences of the major J-C intron immediately 3' of J<sub>c</sub>. The J<sub>c</sub>-2IN primer is 5'-TGAGCCATCGTATTATCCTGAGTCG-3'. It is a 26mer that contains sequences 200 bp 3' of J<sub>c</sub> in the J<sub>c</sub>-2IN intergenic sequence. The J<sub>c</sub>-2IN primer is 5'-AGGGATCCTTGTGAAGGGATCTTACTGTG-3'. It is a 26mer that is entirely contained within the J<sub>c</sub> element and shares no overlapping sequence with the J<sub>c</sub> primer (described above). The oligonucleotides were synthesized on a DNA synthesizer (Applied Biosystems, Foster City, CA) and purified using NENSORB PREP cartridges (Du Pont Co., Wilmington, DE).

**Standard PCR Assay.** PCR amplifications were performed as described (21). Fresh aliquots of reagents were used for each set of experiments. In brief, a set of standard DNAs derived from A-MuLV lines containing known numbers of DJ<sub>4</sub> targets were diluted into DNA that had no DJ<sub>4</sub> targets (CB32, a VDJ/VDJ A-MuLV line). The diluted standards were amplified at the same time as the experimental and control DNAs. A hot start method of the Taq polymerase (Boehringer Mannheim Biochemicals, Indianapolis, IN) being added when the reaction temperature reached 80°C. 30 cycles were carried out. To facilitate the cloning of the larger DJ<sub>4</sub> structures (DJ<sub>4</sub>1 and DJ<sub>4</sub>2), secondary amplifications were performed on 10- and 100-fold diluted primary scid and C.B-17+/+ amplifications, respectively; one of the nested primers (J2-IN or J4-IN) was used with the same cycling conditions.

**Southern Hybridization Analysis.** 10% of each PCR amplification reaction was loaded on a 1% agarose gel (Sigma Chemical Co., St. Louis, MO) and electrophoresis in TAE buffer (23). Gels were Southern blotted on Zeta Probe nylon membrane (Bio-Rad Laboratories, Richmond, CA) using a vacuum blotting system (Vacugene; LKB Pharmacia). Filters were probed with a 32P-labeled J<sub>c</sub>4 probe under conditions previously described (24). Autoradiographs were exposed without intensifying screens either with Kodak film or in a phosphorimager cassette.

**Densitometry.** Densitometry was used to measure the relative intensities of bands on the autoradiographs. Several exposures of Southern blots were scanned using a densitometer (Molecular Dynamics, Sunnyvale, CA). An example of the raw data output for one analysis is shown in Table I. The data were entered in an Mincotsh Excel data base and the number of DJ<sub>4</sub> structures was calculated using the TREND function based on the curves generated by the standard values. The averages of a number of experiments and their standard errors were determined using StatviewII. The values obtained were per microgram of DNA. These values were converted (Table 2) into numbers per organ using the factors: 1 µg of DNA is 1.8 x 10<sup>8</sup> haploid genome equivalents; 2 x 10<sup>7</sup> nucleated cells are in a femur; 1.5 x 10<sup>9</sup> nucleated cells are in a C.B-17+/+ spleen; and 3 x 10<sup>7</sup> nucleated cells are in a scid spleen (25). At times the data from the C.B-17+/+ mice lay outside the standard curve. These estimates were necessarily less accurate.

**Cloning and Sequencing of DJ<sub>4</sub> Probes.** Products derived from the DFS/J<sub>c</sub> primer pair were cloned from amplifications separate from those used for quantification. To prevent contamination, scid and C.B-17+/+ DNA were not amplified at the same time, nor were their amplified products run on the same gel. Negative controls were included with all rounds of amplification. These controls consisted of no DNA and targetless DNA from the 70Z/3 cell line. 70Z/3 has a VDJ and a DQ52/J<sub>c</sub>2 rearrangement, and thus cannot be amplified with the DFS/J<sub>c</sub> primers. Some primary C.B-17+/+ and scid amplifications were cloned directly, and others were cloned after secondary amplification. In these cases, either secondary amplification proceeded directly or primary amplified samples were run on low-melt gels (Nusieve), and plugs, taken from the control and experimental lanes, were amplified in parallel using nested primers. Whenever a product was detected in the negative control lanes, the amplifications were discarded.

The amplified structures were cloned by means of the TA Cloning Kit (Invitrogen) or by the embedded restriction endonuclease sites in the PCR primers. Bacteria harboring positive plasmids were identified by antibiotic selection. Nitrilcellulose membranes (Schleicher & Schuell, Inc., Keene, NH) bearing bacterial DNA were hybridized at 42°C or 37°C overnight with DIG-dUTP-labeled (Biochemistry; Boehringer Mannheim Biochemicals) J<sub>c</sub>2 or J<sub>c</sub>4 probes, respectively. The filters were washed twice with 2 x SSC, 0.1% SDS for 5-min intervals followed by two 1 x SSC, 0.1% SDS washes, at 60°C or 42°C for 30 min. The reduced hybridization and washing temperatures for the J<sub>c</sub>4 probe allowed detection of the 50-nucleotide homology in the DJ<sub>4</sub> structure. To detect the signal, filters were blocked in a solution of 5 x SSC, 5% skim milk solution, 50% formamide, 0.1% N-lauryl sarcosyl, and 0.02% SDS for 3 h and then exposed to an anti-DIG horseradish peroxidase (POD)-conjugated antibody (Biochemistry; Boehringer Mannheim Biochemicals) diluted 1/1,000 in blocking solution for 1 h. The filters were washed in 100 mM Tris, pH 7.5, 150 mM NaCl, and 0.02% Tween20 four times for 15 min each, and then subjected to the enhanced chemiluminescence (ECL) detection.
system (Amersham Corp., Arlington Heights, IL). Positive colonies were selected and plasmid DNA was extracted. Sequencing was performed using the double-stranded method with the T7 Sequencing Kit (Pharmacia Fine Chemicals, Piscataway, NJ); both the reverse and universal sequencing primers were utilized.

Results

Quantification of DJh Structures. To quantitatively assay DJh structures by PCR, we used primers flanking D and Jh4, which results in a “ladder” of DJh1, DJh2, DJh3, and DJh4 when the products are analyzed by gel electrophoresis (Fig. 1a). By simultaneously amplifying standards containing equimolar concentrations of targets of each of the four DJh products, we can estimate their number in tissues. We previously showed that none of these products are preferentially amplified by the DFS/Jh4 primers (21). Fig. 1, b and c, show examples of typical PCR amplifications of DJh rearrangements in BM and spleen DNA from individual scid and C.B-17+/+ mice. There are four discrete bands of sizes corresponding to DJh1, DJh2, DJh3, and DJh4 structures in all samples. The discrete nature of the bands was somewhat surprising due to the paucity and aberrant nature of the scid coding junctions found previously (5, 11). We quantified these products using data derived from seven C.B-17+/+ BM, five C.B-17+/+ spleens, five scid BM, and four scid spleens. One of the scids was serum Ig positive. The raw data from a typical quantification are shown in Table 1; a summary of the quantification data is shown in Table 2. The data are presented as the sum of the four Jh's because in some cases individual scid mice had unequal usage of the Jh segments (Fig. 1, b and c, and Discussion). This unequal usage of Jh was not observed in other

![Genomic map of the DJh locus showing oligomer primer position sites and expected products](image)

**Figure 1.** (a) Genomic map of the DJh locus showing oligomer primer position sites and expected products (20), not drawn to scale. (b) Southern blot analysis of PCR amplification of scid and C.B-17+/+ BM and spleen (SP) DNA using the DFS/Jh4 primer pair. Amplifications of the titration standards were run in parallel. (c) Southern blot analysis of PCR-amplified BM and SP DNA from nonleaky (Ig-) and leaky (Ig+) scid and C.B-17+/+ mice. 14D FL is 14-d C57BL/6 fetal liver DNA amplified for comparison (see reference 21). CB32.12 is a negative control. Titration standards were amplified in parallel as in b.
Table 1. Typical Densitometric Analysis

| DNA source | DJ\textsubscript{1} | DJ\textsubscript{2} | DJ\textsubscript{3} | DJ\textsubscript{4} | Total No./1 \mu g | No. per organ\textsuperscript{0} |
|------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 500T       | cpm             | cpm             | cpm             | cpm             | cpm             | cpm             |
|            | No.\textsuperscript{1} | No.             | No.             | No.             | No.             | No.             |
| 1,000 T    | 113             | 500             | 353             | 500             | 295             | 2,000           |
| 5,000 T    | 743             | 5,000           | 1,750           | 5,000           | 1,860           | 20,000          |
| +/+ BM     | 719             | 4,816           | 1,480           | 4,081           | 1,030           | 2,369           |
| scid BM-17 | 698             | 4,662           | 273             | <500 (101)      | 218             | 6,000           |
| scid BM-19 | 274             | 1,598           | 359             | <500 (385)      | 212             | 4,900           |
| scid BM-1  | 221             | 1,215           | 521             | 919             | 286             | 13,000          |
| scid spleen-18 | 123           | 506             | 154             | <500 (209)      | 91.3            | 1,300           |
| scid spleen-20 | 146           | 672             | 104             | <500 (44)       | 41.9            | 1,400           |

See Materials and Methods for quantification.

\textsuperscript{*} Output on the densitometer.

\textsuperscript{1} Calculation per organ is based on BM having 2 \times 10^7 nucleated cells for all strains; C.B-17+/+ spleen having 1.5 \times 10^7 nucleated cells; scid spleen having 3 \times 10^7 nucleated cells.

Table 2. Quantification of DJ\textsubscript{H} Structure in BM and Spleen

| C.B-17 | scid BM | C.B-17 | scid spleen |
|--------|---------|--------|-------------|
| +/+ BM | scid    | +/+ BM | scid spleen |
|        |         |        |             |
| 1.4 \times 10^6 | 6.7 \times 10^3 | 3.0 \times 10^7 | 2.1 \times 10^7 |
| 1.0 \times 10^6* | 5.4 \times 10^3 | 1.6 \times 10^7 | 2.3 \times 10^7 |
| 2.1 \times 10^6* | 4.3 \times 10^5 | 1.3 \times 10^7 | 5.7 \times 10^7 |
| 6.7 \times 10^6 | 2.8 \times 10^5 | 1.6 \times 10^7 | 1.2 \times 10^7 |
| 3.9 \times 10^6 | 4.8 \times 10^5 | 1.1 \times 10^7 | 1.2 \times 10^7 |
| 4.7 \times 10^6 | 3.0 \times 10^3 | 1.2 \times 10^7 | 1.2 \times 10^7 |
| 1.9 \times 10^6 | 3.9 \times 10^5 |                     |             |
| 2.3 \times 10^6 |                     |                     |             |
| 3.3 \times 10^6 |                     |                     |             |

Average: 3.0 \times 10^6

SE: \pm 0.61 \times 10^6

2 \times 10^7 nucleated BM cells for all strains. 1.5 \times 10^6 nucleated spleen cells for C.B-17+/+ and 3 \times 10^7 cells for scid.

The same samples evaluated in different quantifications.

Each value was determined from a set of data analyzed as detailed in Table 1.
Characterization of DJH Structures. Based on the lack of mature lymphoid cells in scid mice and the aberrant nature of the coding junctions isolated from A-MuLV-transformed lines from scid mice, we had expected to see few, if any, discrete normal-sized DJ1-4 products. To search for more subtle differences in the scid DJH joints, we cloned and sequenced the amplified products and compared them with those from C.B-17+/+. Fig. 2 shows the DJH structures derived from 6- and 27-wk-old BM of C.B-17+/+ mice. Fig. 3 shows the DJH structures from 6-wk-old scid BM. Key features of the analysis are summarized in Tables 3 and 4.

Table 3. Comparison of DJH Structures Derived from scid and C.B-17+/+ BM and BALB/c Fetal Liver (FL)

|                      | scid     | C.B-17+/+ | BALB/c FL |
|----------------------|----------|-----------|-----------|
| No. of structures    | 57       | 57        | 40        |
| analyzed             |          |           |           |
| Average deletions (range) | 8.8 (2-42) | 9.1 (0-41) | 6.7 (2-15) |
| No. of structures    | 14       | 47        | 0         |
| containing nucleotide insertions |          |           |           |
| Average no. of nucleotide insertions for above structures | 5.4     | 4.2       | 0         |
| RF Usage: 1          | 63%      | 44%       | 70%       |
| 2                    | 7%       | 18%       | 8%        |
| 3                    | 30%      | 38%       | 22%       |
| Potentially productive joins | 70%     | 61%       | 90%       |
| DFL 16.1 usage       | 39%      | 81%       | 52%       |

Data are summarized from Figs. 2 and 3. BALB/c FL data are derived from Chang et al. (21).

Table 4. Comparison of DJ1 Structures with and without N or P Nucleotide Additions

|                      | Total scid | Unique scid | Total C.B-17+/+ | Unique C.B-17+/+ |
|----------------------|------------|-------------|-----------------|------------------|
|                      | Total      | Without N or P | Total          | Without N or P | Total          | Without N or P |
| No. of structures    | 57         | 43 (75%)    | 22             | 17 (77%)        | 57             | 9 (16%)        |
| RF: 1                | 63%        | 79%         | 55%            | 59%             | 44%            | 67%            |
| 2                    | 7%         | 2%          | 9%             | 6%              | 18%            | 11%            |
| 3                    | 30%        | 19%         | 36%            | 35%             | 38%            | 22%            |
| DFL16.1 usage        | 39%        | 21%         | 50%            | 32%             | 81%            | 90%            |
| Potentially productive | 70%    | 88%         | 55%            | 71%             | 61%            | 80%            |

Data are summarized from Figs. 2 and 3.
Table 2: DNA sequences of C.B-17+/ mouse. Close designations with a C are derived from 2-week-old C.B-17+/ mouse. The selected distant from or added to the genome sequence, the corresponding elements are indicated. In-frame stop codons are bolded and italicized. Possible point mutations or In-frame stop mutations.
**Figure 3.** DNA sequences of the nonleaky 6-wk-old scid BM DJα structures. A, B, and C designate different scid mice. Lower-case Roman numerals represent separate PCR primary and secondary amplifications. The nucleotides deleted from or added to the germline sequences of the recombining elements are indicated. In some cases deleted nucleotides may have come from either recombining element. Possible P insertions are underlined. In-frame stop codons are italicized and bold. * A DJα structure with a fusion of a partial Jα2 to Jα4. Mutations from germline sequences are indicated by lower-case letters. Clones whose mutations are not shown in this chart have their clone name bold.
tures from C.B-17+/+ were analyzed. Since no difference was observed between structures derived from 6- and 27-wk-old mice, the data in Fig. 2 are pooled from both sources. Deletions were present in most coding joints. An average of 9.1 nucleotides was deleted from D and/or the JH. N and P insertions, which are a common feature in adult Ig gene rearrangement (26), were observed in 82% of the structures, with an average of 4.2 nucleotides being added. Nine structure rearrangement (26), were observed in 82% of the structures, with an average of 4.2 nucleotides being added. Nine structures contain possible P nucleotides, of which seven also have N insertions.

Although Ds elements can be read in all three reading frames (RF) and in either orientation, RF usage is not random. In the C.B-17+/+ BM, RFs 1, 2, and 3 were used in 44, 18, and 38% of the structures, respectively. RF1 is most marked overused in the fetal repertoire where there are few if any N additions due to the lack of terminal deoxynucleotidyl transferase (TdT) activity (27). Terminal homologies of the recombining elements are thought to promote the RF1 usage in cases where there is no N addition (21, 26, 28). Indeed, upon analysis of structures within N nucleotides (nine structures), the RF1 bias becomes apparent: 67:11:22% (Table 4).

Of the 57 C.B-17+/+ structures, 35 (61%) were potentially productive; i.e., they lack stop codons in the used reading frame and invariant residues are present in the JH element. DJ3 structures were the least productive (40%) due to the more frequent usage of RF3, which has many stop codons. The biased D usage reported by us (21) and others (29, 30) was also present; genetic element Dfl16.1 was used in 81% of the DJ3 rearrangements.

DJ3 Rearrangements in scid BM. DNA derived from the BM of three 6-wk-old, Ig- scid mice was individually amplified and cloned. Fig. 3 shows the sequences of 57 DJ3 structures from scid BM DNA, and key features are summarized in Tables 3 and 4.

The scid JH structures shown in Fig. 3 are, in fact, quite similar to those of C.B-17+/+ mice (Fig. 2) and other strains (21, 26, 28). However, there are marked quantitative and qualitative differences, particularly in the degree of diversity. Of 57 scid structures isolated, only 22 were unique; 56 of 57 C.B-17+/+ DJ3 were unique. Moreover, only 14 of the 57 scid structures (5 of the 22 unique ones) contained N or P nucleotide insertions. Of the five unique structures containing N or P, two contained N only, two contained possible P and N, and one structure contained possible P only.

The average number of insertions for structures containing N and/or P was 5.4 (4.4 for unique structures), somewhat more than C.B-17+/+ (4.2 nucleotides [nt]). The mean number of nucleotides deleted from the recombined coding ends was 8.8, which is about the same as C.B-17+/+ (9.1 nt) (Table 3).

scid DJ3 structures used RF1 more frequently than C.B-17+/+. Of the 57 scid DJ3 structures, 63, 7, and 30% used RFs 1, 2, and 3 respectively (compared with 44, 18, and 38%, respectively, for C.B-17+/+). Interestingly, RF1 was used more frequently in both strains when structures joined without N and/or P insertions (Table 4). 70% (55% of the 22 unique structures) of the scid structures could yield a functional Ig protein as defined by the lack of stop codons and the presence of invariant residues in the JH element. None of the scid DJ3 structures were potentially productive. Dfl16.1 was overused, but less frequently than in C.B-17+/+. 39% of the scid (50% if the unique only are considered) structures use this Ds element (Table 4).

Discussion

Functional DJH Joints in scid Lymphoid Progenitors. In A-MuLV-transformed lymphocytes from scid mice, all DJH coding joints were grossly abnormal, containing large deletions of both D and JH regions (5, 9). Therefore, we expected to observe few, if any, DJH joints in cells from scid bone marrow. To our surprise, normal DJH joints were observed in the present study. Moreover, the frequency of progenitor cells containing potentially functional joints was also close to normal. We arrived at this latter conclusion by determining the frequency of normal DJH joints detected by PCR and the proportion of cells in BM belonging to the B cell lineage. In normal BM, ~30% of the cells belong to the B lineage. Of these cells, approximately two-thirds are in the pre-B cell stage (i.e., contain cytoplasmic μ) or B cell stage (i.e., express surface Ig). The remaining cells are in the pro-B stages. According to a recent study by Osmond et al. (31), the early and intermediate pro-B cells in scid mice are normal in frequency and proliferation kinetics. The late pre-B stage is markedly depleted in scid mice, so that overall the number of B lineage cells in the BM of scid mice is only ~10% of the number found in the BM of normal mice. Since scid mice contain ~10% as many DJH joints as normal mice (Table 2), many of the pro-B cells in scid mice must contain potentially functional DJH joints.

These data appear to contradict previously published data on the frequency of normal DJH joints in pro-B cells transformed by A-MuLV (5, 9, 32) and those pro-B cells produced in long-term BM cultures (10, 33) derived from scid mice. In both instances, few, if any, normal DJH joints were observed in cells from scid mice. Several factors may contribute to this discrepancy: The low number of B220+ cells in the BM of scid mice (31) indicates that cells that fail to make a functional heavy chain gene are rapidly deleted in the BM. However, cells with nonfunctional rearrangements may be rescued either by transformation with A-MuLV or by the growth conditions in long-term BM cultures. It is also possible that during the continued growth of these rescued cells the abnormal recombinase system continues to function so that further gross deletions are generated under these two conditions. Thus, the abnormal rearrangements may be a result of the rescue of cells from programmed cell death, and they may not accurately reflect the ability of the recombinase system to carry out DJH recombination.

It is also possible that the major defect in gene rearrangement occurs when the cells attempt to form a Vα-DJH joint. Quasi-normal DJ rearrangements have been reported in scid thymocytes at the δ locus, while there was no evidence of Vδ rearrangement (20). Studies in long-term BM cultures
and in A-MuLV-transformed cells provided little evidence for attempted V to DJ recombination, though it is possible that activation of this process is highly abnormal and leads to the gross deletions mentioned previously. Using primers that detect a large proportion of the Vn joints, we are attempting to investigate VDJn recombination with similar quantitative PCR studies of the type described in this paper.

Implications for the Leaky Phenotype. As mentioned above, scid is a leaky phenotype; normal Ig-secreting lymphocytes arise in scid mice (12). One model put forward to explain leakiness is the somatic reversion model. The premise of this model is that a genetic reversion in a pro-B or earlier cell leads to clones of cells capable of normal gene rearrangement. The major piece of evidence supporting the somatic reversion model is the observation of normal coding joints on the nonexpressed alleles in T cells obtained from leaky scid mice (16). This observation was taken as evidence that the recombination machinery was normal in these cells and hence that a reversion had taken place. Our hypothesis is, instead, that leakiness is the result of the chance occurrence of three functional Ig rearrangements in a single lymphoid cell. Indeed, if a cell ultimately makes a functional receptor (the requirement for leakiness), it is not unlikely that the rearrangement on the other allele is also functional. This explanation would account for the data from Petrini et al. (16), as described above, and does not require that there was a reversion in the recombinase machinery itself.

Fetal Nature of DJn Joints in scid Mice. Although the proportion of potentially functional DJn joints in scid mice was similar to that observed for normal mice, there were unusual features of the joints in scid mice. Very few additional (N or P) nucleotides are inserted into the joint. A similar low frequency is often observed in fetal liver cells (21, 26, 28) and is attributed to the low levels of TdT in fetal liver (27, 28). As reported by Osmond et al. (31), the frequency of TdT+ cells in scid BM is identical to that observed in the BM of normal mice. Thus, the low level of N nucleotides is unlikely to result from overall reduced TdT levels.

The second unusual feature of the DJn rearrangements in scid mice was the restricted repertoire observed. When we sequenced 57 DJn clones isolated from C.B-17/+ + mice, we detected 56 unique sequences. In contrast, the 57 DJn clones from scid mice contained only 22 unique sequences. Six of these sequences were isolated many times. If, as discussed above, the scid mutation results in a dissociation between the timing of gene rearrangement and the sequential transition of cells from pro-B to pre-B to B cells, it is possible that an abnormal expansion occurs in the late pro-B cell stage allowing minor clonal dominance of some DJn rearrangements. Support for this explanation can be seen in some of the Southern blots (Fig. 1, b and c) where, at times, one of the four DJn bands is unusually intense. Such band may represent an expanded clone.

Recently, possible intermediates in V(D)J recombination have been detected in scid thymocyte DNA. These intermediates are "hairpinned" D6 and J6 coding ends. The inability to isolate such structures from normal mouse DNA has lead to the speculation that the scid defect affects the resolution and/or generation of these structures (2, 34). Taken with our finding of a general lack of N insertions in scid, these data suggest that the scid product might interact, or rather interfere, with TdT activity at the stage where the hairpins are resolved. In view of the observations that homologies at the coding ends promote recombination in the absence of TdT (21, 26), examination of DJn joints in scid fetal liver, where recombination occurs solely without N addition, may be informative.

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