MOLECULAR AND CELLULAR BIOLOGY, Oct. 1990, p. 5397–5407
0270-7306/90/105397-11$02.00/0

Vol. 10, No. 10

Wild-Type V(D)J Recombination in *scid* Pre-B Cells

ERIC A. HENDRICKSON,[1] MARK S. SCHLISSEL,[2] AND DAVID T. WEAVER[1]*

Division of Tumor Immunology, Dana-Farber Cancer Institute, and Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115,[1] and The Whitehead Institute and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139[2]

Received 18 May 1990/Accepted 16 July 1990

Homozygous mutation at the *scid* locus in the mouse results in the aberrant rearrangement of immunoglobulin and T-cell receptor gene segments. We introduced a retroviral vector containing an inversional immunoglobulin rearrangement cassette into *scid* pre-B cells. Most rearrangements were accompanied by large deletions, consistent with previously characterized effects of the *scid* mutation. However, two cell clones were identified which contained perfect reciprocal fragments and wild-type coding joints, documenting, on a molecular level, the ability of *scid* pre-B cells to generate functional protein-coding domains. Subsequent rearrangement of the DGR cassette in one of these clones was accompanied by a deletion, suggesting that this cell clone had not reverted the *scid* mutation. Indeed, induced rearrangement of the endogenous kappa loci in these two cell clones resulted in a mixture of *scid* and wild-type V-Jκ joints, as assayed by a polymerase chain reaction and DNA sequencing. In addition, three immunoglobulin μ⁻ *scid* pre-B cell lines showed both *scid* and wild-type V-Jκ joins. These experiments strongly suggest that the V(D)J recombinase activity in *scid* lymphoid cells is diminished but not absent, consistent with the known leakiness of the *scid* mutation.

Functional immunoglobulin and T-cell receptor (TCR) genes are assembled from separate gene elements via somatic gene rearrangement during lymphoid differentiation [V(D)J recombination]. The gene elements targeted for rearrangement are flanked by conserved signal sequences, which mediate the rearrangement event (2, 14, 33). These signal sequences consist of heptamer and nonamer elements separated by a spacer region of either 12 or 23 base pairs (11). Alt and Baltimore (1) proposed that rearrangement is initiated by the recognition of the signal sequences, followed by the introduction of double-stranded breaks at the heptamer-coding element junctures. The subsequent rearrangement event generates two products: a signal junction (also called a reciprocal fragment) and a coding junction. The reciprocal fragment consists of the precise head-to-head joining of the signal sequence heptamers. This rearrangement product will be lost from the chromosome as a circular molecule in the case of deletional rearrangement, whereas it will be retained on the chromosome during inversional rearrangement. In contrast to the reciprocal fragment, the coding junction is marked by a variable loss or addition of nucleotides (N regions).

Mice homozygous for the *scid* (severe combined immunodeficient) mutation lack detectable mature B and T lymphocytes as a result of an apparent V(D)J recombination defect (4, 32). Analysis of Abelson murine leukemia virus (A-MuLV)-transformed *scid* pre-B cells (10, 13, 22, 32), cultured *scid* bone marrow cells (26), and spontaneous *scid* T-cell lymphomas (32) showed that the appropriate gene elements were utilized but that large deletions accompanied the rearrangement events. These deletions removed all or most of the coding sequences (3, 10, 20, 22) and resulted in nonfunctional lymphoid cells incapable of synthesizing immunoglobulin heavy-chain (IgH) or TCR proteins. Hence, we and others have proposed that the *scid* gene product is a component of the V(D)J recombination complex.

We report here studies of continuing gene rearrangement events in *scid* pre-B cells that delineate the effect of the *scid* mutation on V(D)J recombination. We introduced a retroviral construct (DGR) containing an immunoglobulin rearrangement cassette into *scid* pre-B cell lines, allowing direct examination of aberrant *scid* rearrangements from chromosomal templates. Both products of a single rearrangement were determined: coding joints were selectively aberrant, whereas reciprocal fragments were normal. Surprisingly, two sequenced DGR *scid* rearrangements were wild type in all characteristics. To pursue this observation, we used a sensitive polymerase chain reaction (PCR) assay (31) to characterize endogenous kappa light-chain rearrangements in *scid* pre-B cells. These experiments showed clearly that all of the *scid* pre-B cell lines tested contained low levels of recombination activity capable of generating normal kappa locus rearrangements, possibly reflecting the incomplete penetrance of the *scid* mutation. These observations offer one explanation for the diminished state of the immune system in the *scid* mouse: a leaky phenotype generated in part by infrequent wild-type rearrangements.

MATERIALS AND METHODS

Cell culture and recombinant virus infections. A-MuLV-transformed pre-B cell lines derived from either wild-type or homozygous *scid* mice were used. The *scid* cell lines 8D, 2A, and AA2 have defective immunoglobulin gene rearrangements at their IgH alleles (10). The retrovirus DGR (16) was produced from a Ψ2 fibroblast cell line infected with DGR. The *scid* 8D pre-B cell line was infected with DGR by cocultivation with the Ψ2 producers in mass cultures as described previously (10). The mass culture infections were then cloned while maintaining G418 resistance to yield subclones with individual rearrangements. By this protocol, most of the recovered cell clones had undergone recombination events in DGR (Fig. 1; 10). Mycophenolic acid (MPA)-resistant cell lines were isolated either by using an 8D cell line (8-6; Fig. 1) containing an unrearranged DGR cassette or by infecting 8D cells with DGR as described above. In either case, 10^7 cells were selected for 2 weeks in 1 μg of MPA per ml but without G418. At this time,

* Corresponding author.

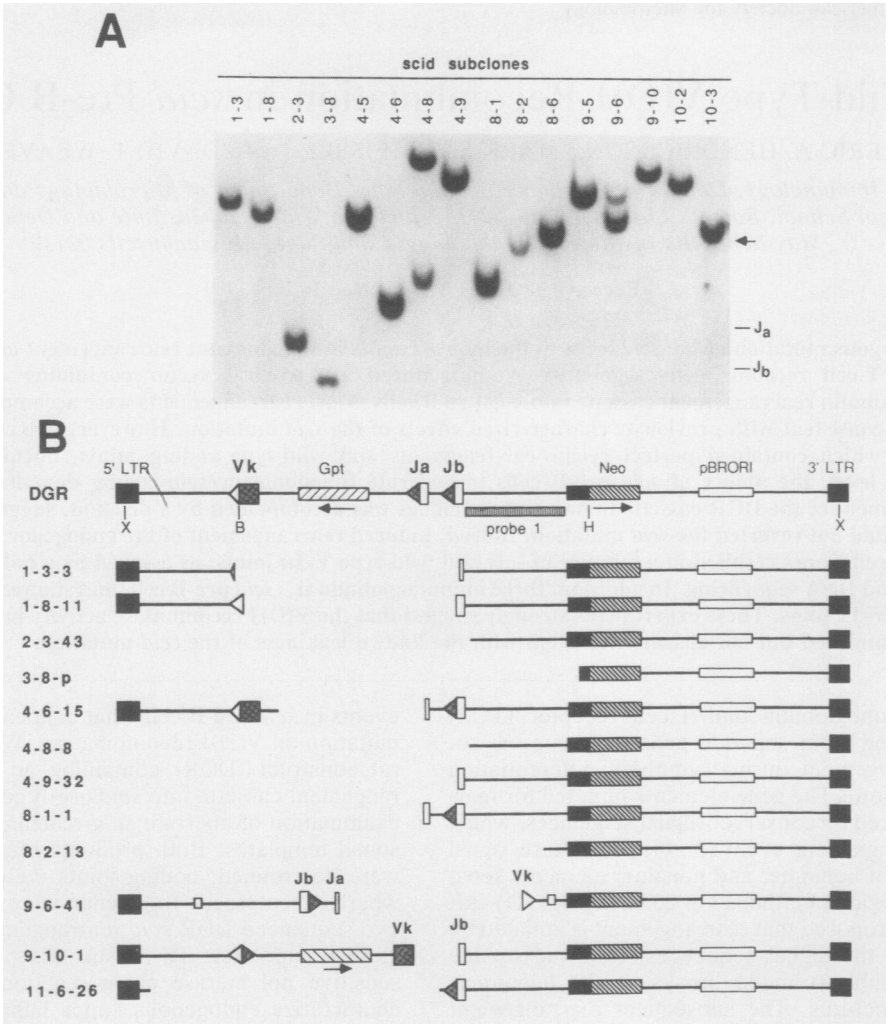

FIG. 1. Aberrant deletional rearrangements of DGR retroviral templates in *scid* pre-B cells. (A) Southern blot analysis of DGR in *scid* subclones. A recombinant retrovirus (DGR) was introduced into *scid* pre-B cells (10). Clones of *scid* cells were established from these infections, genomic DNA was isolated and digested with *Bam*HI plus *Hin*dIII, and a Southern blot was prepared by using probe 1 (pBR322 sequences 972 to 2066) for hybridization. The arrow shows the unrearranged *Bam*HI-*Hin*dIII digest size (3.8 kb) of DGR. Ja and Jb refer to the V-to-Ja and V-to-Jb rearrangements of DGR in wild-type pre-B cells (1.9 and 1.5 kb, respectively; 10, 16). Subclone 8-6 has not undergone any rearrangement and is included as a control. (B) Production of deletions during rearrangement of DGR. The DGR integrated structure consists of 5′ and 3′ LTRs (■); the nonamer-heptamer signal sequence (◁) of Vκ and the Vκ coding sequence (▨); two tandem Jκ1 genes (Ja and Jb; □) flanked by their heptamer-nonamer signal sequences (◀); the *gpt* gene (▨); the simian virus 40 promoter-enhancer (■); the *neo* gene (▨); and the pBR322 origin of replication (pBRORI; □). The hybridization probe 1 position is shown below DGR (▮). The polylinker sequences in clone 9-6-41 are shown by small open rectangles (see text for explanation). The transcriptional orientations of the *neo* and *gpt* genes are indicated by arrows. Relevant restriction enzyme sites are *Bam*HI (B), *Hin*dIII (H), and *Xba*I (X). Genomic DGR DNA from the various *scid* cell lines was cloned (see Materials and Methods) and analyzed by extensive restriction mapping, and deletion breakpoints were determined by DNA sequencing. DGR DNA clone designations are shown at the far left. The first two numbers of each clone correspond to the cell line from which it was isolated (A). Gaps in the DGR map for each clone represent the absence of these DGR sequences. It should be noted that the clones recovered from the 3-8 and 9-10 cell lines (3-8-p and 9-10-1, respectively) do not correspond to the predominant band observed in the Southern blot (A). These clones presumably represented submolar species that were isolated instead of the major rearrangement.

individual clones were recovered from 96-well plates and expanded for analysis (see below).

Southern blots. Procedures for preparation of genomic DNA and Southern blot analysis have been described (10).

Recovery of DGR templates from genomic DNA. Since the DGR-integrated recombination substrate contains a pBR322 origin of replication and the neomycin phosphotransferase (*neo*) gene, it will grow as a plasmid under kanamycin selection in *Escherichia coli* (16). From selected DGR-containing cell lines, 30 μg of genomic DNA was digested to completion with *Xba*I and fractionated on a 0.8% preparative agarose gel in TAE buffer (23). Size fractions were cut from the gel according to the corresponding sizes of rearranged bands observed on previous Southern blots (data not shown) and then isolated from agarose by the glass bead-NaI procedure (34). Approximately 200 ng of size-fractionated DNA was ligated in a volume of 200 μl under standard conditions, transformed into *E. coli* MC1061 by the procedure of Hanahan (9), and plated under kanamycin selection for expression of the neomycin resistance gene. Colonies were analyzed by DNA minipreps, restriction digests, and DNA sequencing.

DNA sequencing analysis of DGR rearrangements. Exact recombination breakpoints were mapped by DNA sequencing. Oligonucleotides from several defined regions of the DGR template were synthesized for this DNA sequencing analysis. The locations of the primers are listed according to a 5′-to-3′ orientation of DGR (Fig. 1). Primer 1065 (20-mer; GCCGGAAGCGAGAAGAATCA) was located ~40 nucleotides (nt) 3′ to Jb; primer 1064 (20-mer; TAAGTGCGGC GACGATAGTC) was located ~65 nt 3′ to Ja; primer 904 (16-mer; GCCAGGTTCAGTGGCA) was located in Vκ coding sequences 94 nt 3′ to the heptamer; primer 903 (16-mer; GCGCTATATGCGTTGA) was located ~110 nt 5′ to the Vκ heptamer. In the DNA sequencing of DGR inversional rearrangements, primers 1065, 1064, and 904 were used to identify V-to-J coding joints. Primer 903 was used to characterize reciprocal fragment junctions. All DNA sequencing reactions were carried out according to Sanger et al. (29), using the Sequenase kit (U.S. Biochemical Corp.).

Assay of kappa locus DNA rearrangements. *scid* pre-B cell lines 8D, 2A, and AA2 and T9.1 subclones 8, 9, 11, and 12 were grown in culture for 7 to 9 days with or without lipopolysaccharide (LPS; 10 μg/ml). Genomic DNA was prepared from 10^6 cells per cell culture, and then the PCR was conducted on 1/100 of each genomic DNA preparation (10^4 genomes), using a Jκ2-Jκ3 region oligonucleotide (JκB; see below) and a degenerate oligonucleotide mixture homologous to Vκ coding sequences at amino acids 60 to 75 according to the procedure of Schlissel and Baltimore (31). The PCR products were fractionated on 1.2% agarose gels for either Southern blots or preparative isolation. To clone putative light-chain rearrangements, a 150- to 750-nt size fraction was excised, and the DNA was purified by the glass bead-NaI procedure (34). This size fraction was large enough to include normal V-to-Jκ1, normal V-to-Jκ2, and aberrant-size rearrangements from the *scid* cells. The DNA samples were treated with ATP and polynucleotide kinase and then ligated with 50 ng of a *Sma*I-linearized pCU19 plasmid vector (23). After transformation into DH5 cells, colonies containing cloned V-to-J rearrangements were detected by colony hybridization with a 1.8-kilobase (kb) [^{32}P]DNA probe (*Hin*dIII-*Bgl*II) spanning the Jκ region (see Fig. 5). Miniprep plasmid DNA procedures were then used to identify either V-to-Jκ1 or V-to-Jκ2 rearrangements by approximate insert size. DNA sequencing of miniprep DNA was conducted by using either the Universal primer of pCU19 (New England BioLabs, Inc.), primer 502 (20-mer; CCACA GACATAGACAACGGA), located in the Jκ1-Jκ2 interval 64 nt from the Jκ1 heptamer, or primer JκB (25-mer; CCTCT TGTGGGACAGTTTTCCCTCC), located in the Jκ2-Jκ3 interval 156 nt from the Jκ2 heptamer. In addition, the degenerate Vκ primer mix was used in DNA sequencing to identify several of the V-to-Jκ2 rearrangements.

RESULTS

***scid* pre-B cells aberrantly rearrange an integrated recombination substrate.** A-MuLV-transformed *scid* pre-B cells were infected in mass culture with DGR, a recombinant retrovirus containing an immunoglobulin rearrangement cassette (Materials and Methods; Fig. 1B; 10). The cassette consisted of a Vκ gene with flanking heptamer-nonamer sequences, in an inverted orientation relative to two tandem copies of the Jκ1 gene (Ja and Jb) and their heptamer-nonamer sequences. Wild-type pre-B cell lines promoted a characteristic inversional rearrangement of the DGR cassette, forming two new junctions: the signal or reciprocal fragment (a precise head-to-head fusion of two heptamer-nonamer sequences) and the coding junction (V-Jκ) (10, 16, 30). Single-cell *scid* subclones were derived from mass infection cultures that had been selected in G418 by using expression of the *neo* gene in DGR. *neo* expression from an internal simian virus 40 promoter-enhancer was independent of immunoglobulin DNA rearrangement in the DGR cassette. The frequency of G418-resistant clones obtained from *scid* cultures was similar to the frequency of clones obtained after DGR infection of wild-type A-MuLV-transformed pre-B cell lines (E. A. Hendrickson and D. Weaver, unpublished observations).

Genomic DNA isolated from the *scid* DGR subclones was digested with *Bam*HI and *Hin*dIII and subjected to a Southern blot analysis using hybridization probe 1 (Fig. 1A). Most of the subclones appeared clonal, whereas a few of the subclones (e.g., 4-8 and 9-6) had clearly undergone two or more independent rearrangements. Each of the subclones had rearranged DGR aberrantly, producing novel *Bam*HI and *Hin*dIII fragment sizes different from those expected with normal V-to-Ja and V-to-Jb rearrangements. In addition, the *scid* genomic DNAs were digested with *Xba*I, which cuts in the viral long terminal repeats (LTRs) of DGR, and then hybridized with probes at different positions in the 5′ half of DGR. Most of the *scid* DNA rearrangements lacked any sequences hybridizing to these other probes, suggesting that they had sustained extensive deletions in the DGR cassette (data not shown).

We cloned the *scid* DGR rearrangements from most of the subclones to delineate the new breakpoints formed (Fig. 1B; see Materials and Methods). Most frequently, the deletions removed the entire 5′ side of DGR (~4 to 5 kb) and extended an unknown distance into mouse flanking genomic sequences, whereas the 3′ side containing the *neo* gene was always retained because of the G418 selection scheme. However, DGR was observed to incur deletions of up to 1.0 kb 3′ from Jb without disrupting *neo* expression (e.g., clone 8-2-13). Thus, continuing rearrangement of DGR in *scid* pre-B cells was aberrant, and the deletional events observed were analogous to the endogenous IgH region deletions characterized in *scid* pre-B cells (10, 32).

Interestingly, some of the clones showed hallmarks of wild-type rearrangements. The inversion in clone 9-10-1 contained a perfect reciprocal fragment. However, an aberrant 462-nt deletion was found at the V-Jκ coding joint. This deletion was clearly initiated from the Ja heptamer element during rearrangement because the reciprocal fragment was formed with the Ja heptamer-nonamer. The deletion was also unusual in that the loss of nucleotides occurred on only one of the two coding strands. Two clones (1-8-11 and 9-6-41; Fig. 1B) were characterized to have hybrid junctions, first described in wild-type (19, 24) and subsequently in *scid* (20) V(D)J recombination, that are defined as reciprocal rearrangements in which the wrong two strands are joined; e.g., a signal sequence of a V element joins with a J coding element instead of to another signal sequence. Clone 1-8-11 joined the Vκ heptamer-nonamer signal with the Jb gene with the loss of 0 and 2 nt, respectively, and had an N-region addition of AG in the hybrid joint. Clone 9-6-41 similarly joined the Vκ heptamer-nonamer signal to the Ja gene with the loss of 0 and 21 nt, respectively. Clone 9-6-41, as determined by DNA sequencing, appeared to have undergone this immunoglobulin-related rearrangement after undergoing an inversion mediated by homologous recombination between two identical polylinker sequences (Fig. 1B; data not shown). Since this event did not involve immuno-

globulin gene rearrangement signals, its significance was not pursued. In addition, the deletion in clone 1-3-3 might also have arisen by a hybrid joint rearrangement. In this case, however, 25 nt was deleted from the Vκ heptamer-nonamer signal and 35 nt was deleted from the Jb coding sequence. We also identified a DGR deletion that removed only a single heptamer-nonamer signal sequence along with flanking DNA (clone 4-6-15; Fig. 1B). This type of *scid* rearrangement will be discussed in more detail in another report (E. A. Hendrickson, V. Liu, and D. Weaver, unpublished data). Together, these experiments showed that most DGR rearrangements in *scid* cells were aberrant as a result of large deletions that removed part or all of the immunoglobulin cassette. The preservation of some of the features of normal inversional rearrangements in several of the *scid* clones was indicative of many similarities with wild-type rearrangement events.

Coding-joint formation is selectively aberrant in *scid* rearrangements. The heptamer-nonamer and coding-junction regions were usually lost during *scid* rearrangements, making it difficult to assess whether the formation of one or both of the recombination junctions was primarily affected. We took advantage of the inversional rearrangement configuration of DGR and a second selection scheme to recover joining events in *scid* cells in which all products of a single rearrangement (a signal junction and a coding junction) are retained on the same region of chromosomal DNA (16). The *gpt* gene of DGR, located between the Vκ and Jκ elements, is inactive without rearrangement. In the rearranged or inverted orientation, *gpt* is transcribed and can be selected for growth in MPA (16, 17). Since neither the exact formation of the reciprocal fragment nor the coding joint is required for *gpt* expression, inversions of DGR in *scid* pre-B cells could be selected for if the extent of the *scid* deletions were limited.

scid pre-B cells containing integrated and unrearranged DGR proviruses were placed in MPA selection without G418, and seven MPA-resistant *scid* cell lines were obtained. Genomic DNA was isolated, digested with *Bam*HI and *Hin*dIII, and subjected to Southern analysis using hybridization probe 1 (Fig. 2A and C). All of the lines displayed appropriate DGR gene rearrangements of 1.9 or 1.5 kb, indicating joining of Vκ to either Ja (clones 8-1, 8-5, 8-10, T5.3, and T9.1) or Jb (clones 8-2 and 8-7), as determined by comigration with wild-type V-to-Ja and V-to-Jb controls (DGR-38B9). When these same DNAs were cleaved with *Xba*I and analyzed by Southern analysis using probe 1 (Fig. 2B), they all generated the full-length *Xba*I fragment expected for normal immunoglobulin inversional recombination in DGR. Thus, at the level of Southern blot analysis, there was at most very limited deletion associated with these selected rearrangement events in *scid* cells.

DGR DNAs were recovered from these cell lines, and the structures of the two recombination junctions were determined by DNA sequencing (Fig. 3). Clone 9-10-1 (Fig. 1B) was included in Fig. 3, since it had similarly undergone an inversional rearrangement, albeit not under MPA-selective conditions. As in wild-type rearrangements (18), the reciprocal joints formed perfectly, with no addition or deletion of nucleotides, in each of the seven cases of *scid* rearrangement events. Interestingly, the coding joints of these same recombination events were frequently aberrant. In five of seven cases examined, the *scid* coding-joint deletions (ranging from −9 to −462 nt) were more extensive than normal for wild-type V(D)J recombination junctions. By analogy to DGR inversional rearrangements (18) and endogenous events in wild-type pre-B cells (12), deletions of larger than

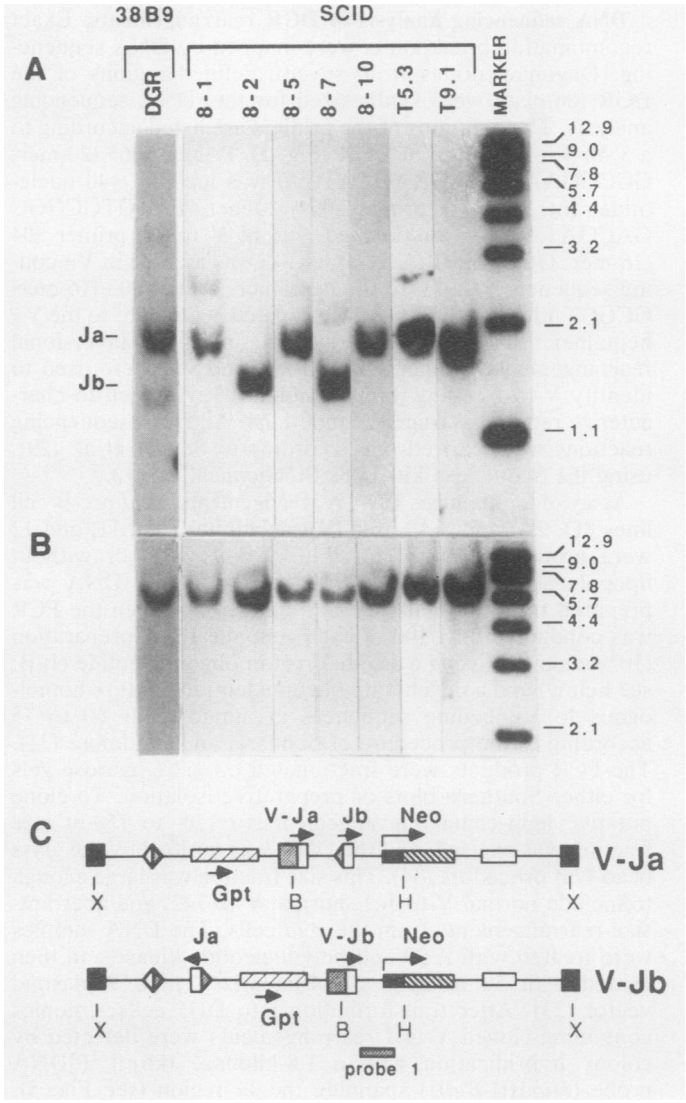

FIG. 2. Detection of DGR inversional rearrangements in *scid* cells. *scid* (8D) and wild-type (38B9) pre-B cell lines were infected with DGR, and MPA-resistant colonies were selected. The rearrangement structures of DGR DNA in these cell colonies were examined by Southern blot analysis using hybridization probe 1. (A) *Bam*HI-*Hin*dIII restriction digestion and Southern blot. The expected wild-type Vκ-to-Jκ rearrangements are indicated on the left (Ja, 1.9 kb; Jb, 1.5 kb). There are two bands in the 38B9 lane because this sample contained a mixture of recombinants. Molecular weight standards (marker lane) are shown on the right in kilobases. (B) *Xba*I restriction digestion and Southern blot analysis with probe 1. (C) Structure of V-to-Ja or V-to-Jb inversional rearrangements of DGR. Symbols are as described in the legend to Fig. 1.

5 nt on one or both coding elements were defined as aberrant. In these aberrant coding joints, we observed that deletion occurred from both the Vκ gene (−9 to −31 nt) and the Ja or Jb gene (−13 to −462 nt). In two cases (9.10.1 and 8.2), the Vκ gene was not deleted, whereas the Ja or Jb gene was deleted substantially. We found that four of seven of the clones contained N regions in the coding junctions (Fig. 3). These data demonstrated that the *scid* mutation primarily affected the formation of coding joints in a rearrangement event in which all recombination products from the event were identifiable.

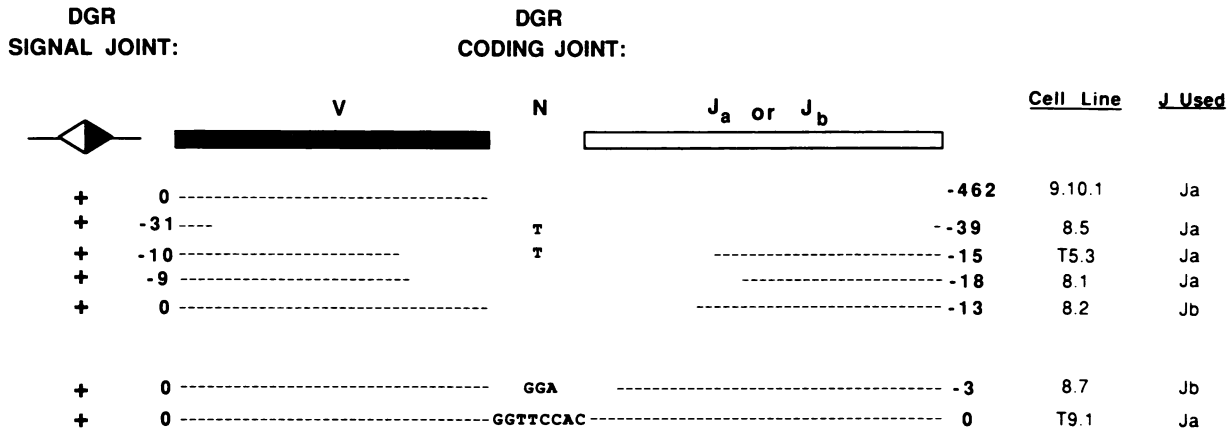

FIG. 3. Demonstration that formation of the coding junction is selectively aberrant. The rearranged DGR templates from the cell lines in Fig. 2 were molecularly cloned, and the breakpoint junctions were identified by DNA sequencing (see Materials and Methods). DGR signal joint: + indicates a wild-type reciprocal fragment junction without the deletion or addition of nucleotides. A wild-type reciprocal fragment is an exact fusion of two heptamer-nonamer elements of opposite spacer length (represented by triangles; see DGR structure in Fig. 1). DGR coding joint: The nucleotide sequence of the V and Ja or Jb elements are represented as boxes in a rearranged coding-junction configuration. Dashed lines indicate nucleotides retained in the *scid* inversions, and blank spaces indicate the nucleotides deleted. Numbers flanking these lines indicate the extent of deletions for V or J coding regions. N-region addition (N) in the coding joint is shown. The type of inversion rearrangement detected, V-to-Ja or V-to-Jb, is listed on the right.

Surprisingly, two *scid* inversions were indistinguishable from wild-type rearrangements (T9.1 and 8.7; Fig. 3). Both clones had a perfect reciprocal junction and either no deletion or a small deletion in the coding joint. Both clones had N regions in the coding joints. Because all of these *scid* cells were derived from the same starting clone (8D) which had characteristic aberrant endogenous immunoglobulin gene rearrangements (10), we can conclude that these wild-type DGR recombination events occurred after phenotypically *scid*-type rearrangements.

Secondary rearrangements in clone T9.1 are aberrant. One property of *scid* mice is that a sizeable fraction of advanced-age animals acquire a limited B- and T-cell repertoire (leaky *scid* mice; 5). A low level of normal rearrangements, such as that observed with DGR, provides one model for the leaky phenotype seen in *scid* animals. Alternatively, true genetic reversion of the *scid* mutation might occur frequently enough in the proliferation of *scid* lymphoid precursor cells to account for the limited B- and T-cell repertoire. We experimentally tested these two models, using the T9.1 *scid* clone, by characterizing continuing rearrangements of the T9.1 provirus.

DGR with one wild-type V-to-J junction can undergo secondary rearrangement by using the heptamer-nonamer of the unrearranged J element with the Vκ signal sequence of the reciprocal fragment (18). A correct second event inverts the *gpt* gene to its original orientation, which causes the cells to become MPA sensitive, and changes the hybridizing *Bam*HI-*Hin*dIII fragment size from 1.9 to 3.35 kb. We examined secondary rearrangements by subcloning T9.1 in nonselective medium. Genomic DNA prepared from 24 subclones was subjected to a Southern analysis with hybridization probe 1 after *Xba*I or *Bam*HI-*Hin*dIII restriction digests (Fig. 4A; data not shown). Since *Xba*I cuts uniquely in the LTRs, the size of T9.1 DGR DNA (~7.0 kb) should be unchanged by internal rearrangements unless they are accompanied by deletions. Twelve subclones showed the parental T9.1 size for both digests (e.g., T9.1-8; Fig. 4A), suggesting no additional rearrangement. Ten subclones had an identical aberrantly sized hybridizing fragment (e.g., T9.1-7; Fig. 4A), indicating a *scid* deletion event prior to the subcloning step. One subclone (T9.1-6) no longer contained sequences homologous to probe 1, suggesting that it too had sustained a large *scid*-like deletion (data not shown). Additional mapping of this deletion was not performed. Finally, we found one example (T9.1-23) that had the appropriate *Bam*HI-*Hin*dIII size (3.35 kb) and *Xba*I size (7.0 kb) to potentially be a wild-type secondary rearrangement (Fig. 4A).

We cloned the DGR DNA for subclones T9.1-7 and T9.1-23 to determine the DNA sequences across the new rearrangement breakpoints. In the T9.1-7 secondary rearrangement a hybrid joint was generated, consisting of a 2-nt deletion of the Vκ signal sequence and a 10-nt deletion of Jb coding sequence (Fig. 4B and C). In contrast, both a new reciprocal junction and a new coding junction were formed in the secondary DGR rearrangement of T9.1-23. However, both new junctions were found to be aberrant by DNA sequencing. The new reciprocal joint brought together the heptamer element from Vκ and the heptamer element from Jb, but the heptamer of Jb had been deleted by 2 nt (Fig. 4C). Also, 12 nt had been deleted from the Ja heptamer-nonamer sequence, and 20 nt was deleted from the Jb coding sequences in forming a new coding joint. These observations indicated that these T9.1 subclones had made aberrant secondary rearrangements and had not genetically reverted the *scid* mutation.

Induced rearrangements in T9.1 subclones retain the *scid* phenotype. We examined the potential reversion phenotype more rigorously in clone T9.1 by sequencing endogenous light-chain gene rearrangements that occurred in tissue culture after the DGR rearrangement. T9.1 subclones that had not participated in secondary DGR rearrangement were induced to undergo endogenous immunoglobulin kappa light-chain Vκ-to-Jκ rearrangement in culture by treatment with LPS. LPS treatment stimulates increased transcription in the vicinity of the κ light-chain J-C region (25) and was recently shown to increase the frequency of Vκ-to-Jκ rearrangements (31). New V-to-Jκ rearrangements were detected with a PCR assay capable of detecting quantitative

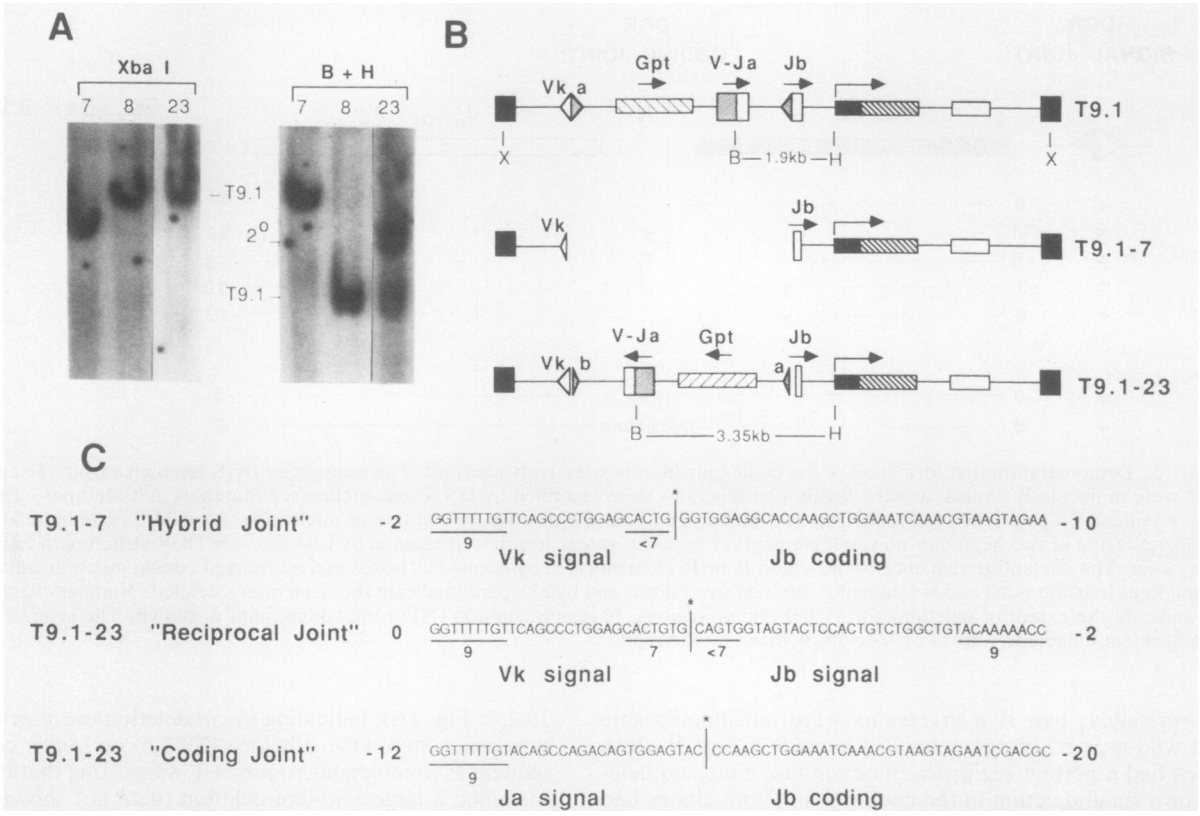

FIG. 4. Aberrant secondary rearrangements follow wild-type primary DGR rearrangements in *scid* pre-B cells. The *scid* clone, T9.1, containing a wild-type DGR rearrangement, was subcloned to analyze continuing secondary rearrangements in the DGR template. (A) Southern blot of genomic DNA of specific subclones of T9.1 digested with *Xba*I or *Bam*HI plus *Hin*dIII (B + H) and hybridized with probe 1 (Fig. 1). 2^0, a 3.35-kb B + H fragment expected for a wild-type secondary rearrangement in DGR. T9.1, a 7.0-kb *Xba*I or a 1.9-kb B + H fragment of the T9.1 DGR containing no additional rearrangement. (B) Structure of DGR secondary rearrangements in T9.1-7 and T9.1-23 subclones. All symbols are the same as for DGR in Fig. 1. (C) Determination of the DNA sequences of the novel breakpoints for T9.1-7 and T9.1-23. The number of nucleotides deleted from each strand is shown on the left and right, respectively. *, Breakpoint position.

differences in the frequency of κ-gene rearrangement (Fig. 5A; Materials and Methods; 31). The J-region primer was located between Jκ2 and Jκ3 and therefore detected either V-to-Jκ1 or V-to-Jκ2 rearrangements. The Vκ primer mix, degenerate at 4 of 31 positions, hybridized to 80% of the known mouse Vκ genes. PCR reactions on genomic DNA samples from four T9.1 subclones (8, 9, 11, and 12) grown in the presence or absence of LPS for 1 week were fractionated and analyzed on Southern blots probed with a restriction fragment from the Jκ region (Fig. 5B). For the four T9.1 subclones, LPS treatment led to a greater than 10-fold increase in the level of V-to-Jκ gene rearrangements detected before induction (compare lanes − versus +). Also, the two predominant sizes of V-to-Jκ1 and V-to-Jκ2 rearrangements (600 and 250 nt, respectively) were seen much more abundantly than any aberrant sizes from the PCR reactions, suggesting possible wild-type V-to-Jκ coding junctions.

The precise nature of these V-to-Jκ rearrangements was determined by DNA sequencing of cloned PCR products (Materials and Methods). In order not to bias the data toward wild-type rearrangements, the entire V-Jκ1-2 region (150 to 750 nt) was excised from a preparative gel for cloning. We characterized 23 unique V-to-Jκ rearrangements; 17 had deletions in the coding junctions that were larger than 5 nt (Fig. 6A). We have arbitrarily defined *scid* deletions as those exceeding 5 nt, even though occasional deletions of 6 to 12 nt have been observed with wild-type rearrangements (12). In contrast, six of six κ rearrangements characterized by this assay using wild-type pre-B cells showed normal V-to-Jκ coding junctions with deletions of less than 6 nt (M. Schlissel, unpublished observations). Generally, aberrant *scid* deletions were identified in both the Vκ and Jκ coding sequences. The exact size of a given deletion of Vκ was uncertain by 0 to 3 nt because the corresponding Vκ germ line sequences were not specifically known. The deletions observed occurred in rearrangements to both Jκ1 and Jκ2; as expected from the Southern blot, Jκ1 was represented almost four times as frequently as Jκ2. In addition, almost all of the Vκ-Jκ rearrangement events were unique, as determined by DNA sequencing of the Vκ region (data not shown). In-frame rearrangements were observed 38% of the time, in keeping with random, nonselected rearrangements.

Surprisingly, 6 of the 23 V-to-Jκ rearrangements were indistinguishable from wild-type joining events (Fig. 6B). Each of the four subclones (T9.1-8, -9, -11, and -12) showed a mixture of *scid* and wild-type V-to-Jκ junctions. In this group, two of the V-to-Jκ rearrangements had N-region additions. Collectively, these data showed that the T9.1 cell lines retained the *scid* phenotype but that wild-type events could be detected readily.

Infrequent wild-type rearrangements are a property of *scid* cell lines. Three independent *scid* pre-B cell lines were also

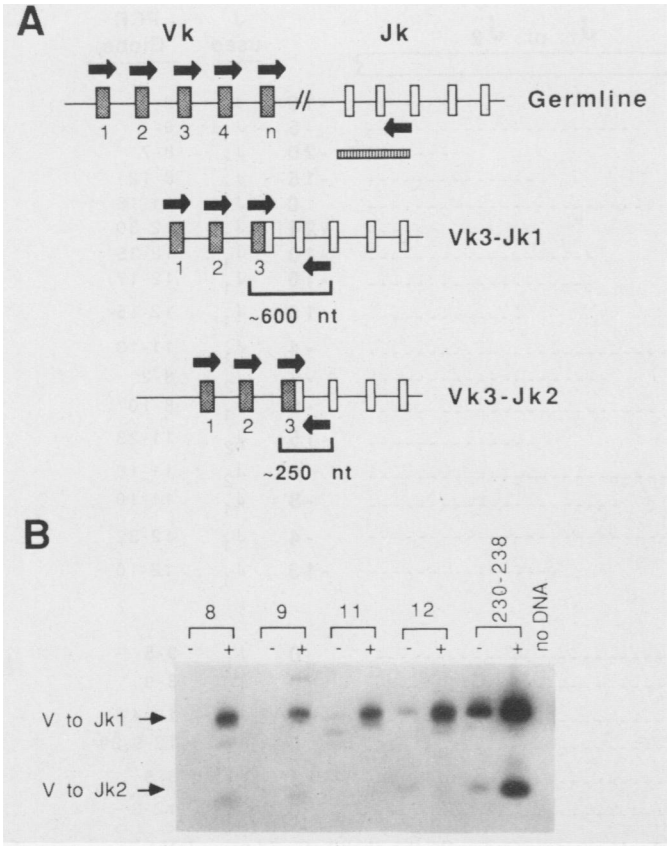

FIG. 5. Induced kappa light-chain rearrangements in *scid* pre-B cells. (A) PCR assay for light-chain gene rearrangement. Two primers (arrows indicate 5′-to-3′ orientation) are matched for the PCR. One primer is a degenerate mixture of oligonucleotides hybridizing to an internal region present in 80% of Vκ genes. The other is a specific primer hybridizing to the interval between Jκ2 and Jκ3 (see Results and Materials and Methods). Vκ-to-Jκ rearrangement brings one of the Vκ genes into close proximity to the position of the Jκ primer, since almost all Vκ-to-Jκ rearrangements are to Jκ1 or Jκ2. Vκ-to-Jκ1 and Vκ-to-Jκ2 rearrangements should generate PCR product sizes of approximately 600 and 250 nt, respectively. ▥, A 369-nt *Rsa*I fragment used as a hybridization probe for Southern blots. (B) Southern blot of PCR products from *scid* pre-B cell lines cultured in the presence (+) or absence (−) of LPS. Cell lines 8, 9, 11, and 12 are *scid* T9.1 subclones; 230-238 is a wild-type pre-B cell line. No DNA indicates a control PCR performed with no added DNA. The positions of DNA fragments corresponding to V-to-Jκ1 and V-to-Jκ2 rearrangements are marked on the left.

examined by PCR for the presence of wild-type endogenous κ-gene rearrangements. Each of these cell lines contained two characterized defective endogenous IgH gene rearrangements (8D, 2A, and AA2; 10). Endogenous light-chain gene rearrangement was stimulated as described above, and Vκ-to-Jκ gene rearrangements were analyzed in an identical fashion by PCR, Southern blots, cloning, and DNA sequencing. Southern blots of 8D, AA2, or 2A κ rearrangements showed V-to-Jκ1 and V-to-Jκ2 sizes predominantly (data not shown). In addition, as had been observed with the T9.1 subclones, κ rearrangements in the absence of LPS were also detected (lanes − of Fig. 5B; data not shown). A DNA sequence analysis of the novel V-to-Jκ coding junctions demonstrated that the LPS-stimulated *scid* cell lines generated predominantly *scid* light-chain rearrangements (19 of 26; Fig. 7A). However, a substantial fraction (7 of 26) had wild-type V-to-Jκ junctions, including the addition of N regions (Fig. 7B). Thus, the appearance of wild-type rearrangements was not unique to T9.1 or other *scid* cells that had undergone one previous wild-type rearrangement.

DISCUSSION

***scid* pre-B cells exhibit a low level of wild-type V(D)J recombination events.** Mice homozygous for the *scid* mutation are severely deficient in B and T lymphocytes (4, 7), yet the defect in lymphopoiesis is not absolute, since 15% of 3- to 4-month old *scid* mice produce serum immunoglobulin and higher percentages are observed with advancing age (leaky mice; 5). Two possible mechanisms for the appearance of immunoglobulin are (i) a genetic reversion of the *scid* mutation to wild type and (ii) a *scid* V(D)J recombination system that is functionally normal a fraction of the time. These explanations are not mutually exclusive. We have shown here that *scid* lymphoid pre-B cells contain a low level of wild-type V(D)J recombinase activity. The retroviral vector DGR was introduced into *scid* pre-B cells, and a drug selection scheme was used to isolate two cell lines (8.7 and T9.1) in which DGR had undergone wild-type inversional rearrangement. These events were indistinguishable from wild type in that they contained perfect reciprocal fragments, had no or small (<6-nt) deletions in their coding joints, and showed the addition of N-region nucleotides. The finding of aberrant secondary rearrangements of DGR in T9.1 subclones clearly showed that genetic reversion of the *scid* mutation had not taken place. To determine whether the endogenous κ locus behaved in an analogous fashion, four T9.1 subclones and three independent *scid* pre-B cell lines were stimulated to undergo κ light-chain gene rearrangement by treatment with LPS. Analysis of these gene rearrangements revealed that the *scid* phenotype was usually produced, but a significant fraction of the events were wild type. In summary, we could find no differences between the T9.1 cell line preselected for a wild-type DGR rearrangement and three unselected *scid* A-MuLV-transformed cell lines. Thus, we conclude that these events were not the result of reversion of the *scid* phenotype but were due to low levels of wild-type V(D)J recombination.

How rare are wild-type gene rearrangements in *scid* cells? The seven MPA-resistant clones that we obtained arose at a frequency 100 times lower than that with which clones were recovered from the wild-type pre-B cell line, 38B9 (Hendrickson and Weaver, unpublished observations). Because of limitations of the DGR selection procedure, in which rearrangements accompanied by deletions that remove sequences necessary for *gpt* expression could not be scored, we were unable to determine whether this 100-fold difference was due to an overall reduction of recombination frequency or due to the high percentage of aberrant rearrangements in *scid* cells. Our analysis of kappa light-chain rearrangements, however, provided us with the opportunity to establish a minimum frequency for wild-type gene rearrangements in *scid* cells. Considering that 10^4 genomes were amplified in our standard PCR assay and about two unique, wild-type V-Jκ junctions were identified per reaction, the frequency of wild-type rearrangements per total rearrangements was estimated to be at least 10^{-4}. Since this calculation assumes that both κ loci had rearranged on every genome (an unlikely scenario), we view this as a minimum estimate. This frequency may be significant in explaining the phenotype of leaky *scid* mice.

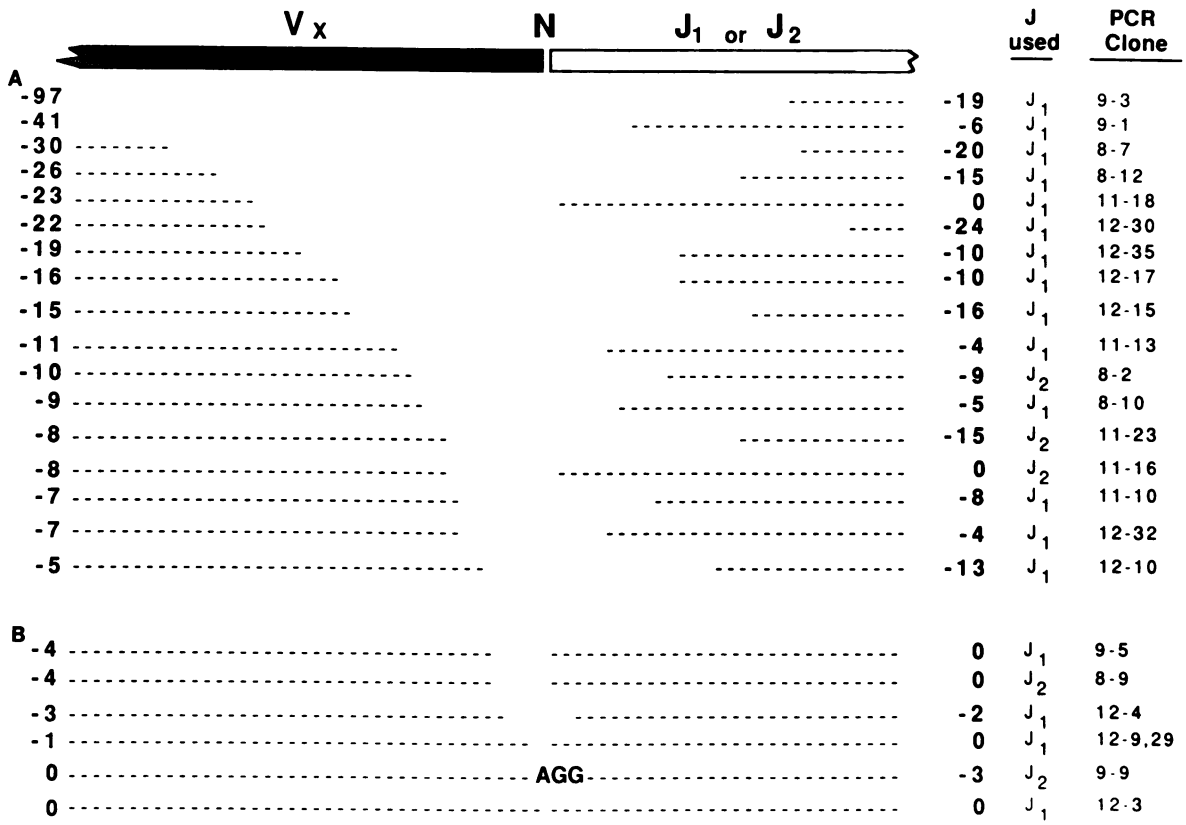

FIG. 6. Demonstration that LPS-induced light-chain gene rearrangements are predominantly the aberrant *scid* type. DNA sequences of V-to-J coding junctions were determined for LPS-induced light-chain rearrangements in the *scid* clone T9.1 (see Fig. 5 and Materials and Methods). Vx represents any Vκ gene used in the rearrangement detected by PCR; rearrangements to either Jκ1 or Jκ2 are specified. Negative numbers refer to the extent of deletion into either the Vκ or Jκ coding sequence from the flanking heptamer border in the formation of the joint. The exact size of a given deletion of Vκ was uncertain by 0 to 3 nt because the corresponding Vκ germ line sequences were not always specifically known. One clone, 9-9, had an N-region addition of 3 nt.

It is interesting that the rearrangements recovered contained deletions which were generally considerably smaller than the size limits which either assay would have allowed. In the case of DGR substrates selected with MPA, deletions of 0.9 kb 5′ and 1.7 kb 3′ to the V-Ja coding junction could have occurred without altering the selection scheme, yet the median DGR deletion was only 43 nt in the coding joints. Similarly, deletions of approximately 100 nt into Vκ and 510 nt from Jκ1 would still have been detected in our PCR protocol. As is evident from the Southern blots (Fig. 5) and DNA sequencing data (Fig. 6 and 7), the average deletion is quite small and only slightly different from wild type. In sharp contrast, all of the unselected DGR deletions (Fig. 1) and all of the chromosomal rearrangements analyzed (3, 10, 13, 22, 32) often contained deletions on the order of several kilobases. This bimodal distribution in the size of *scid* deletions suggests that there may be two steps or two different mechanisms for the joining of broken chromosomal ends generated during the process of lymphoid gene rearrangement. If this scenario is correct, then one of the pathways is clearly more sensitive to the effects of the *scid* mutation. Analysis of more *scid* rearrangement events will help in addressing this issue.

Coding joints are selectively affected by the *scid* mutation. Characterization of endogenous immunoglobulin μ, and κ, and TCR β loci in *scid* lymphoid cells has revealed that large deletions are characteristic of most chromosomal gene rearrangements (3, 10, 13, 22, 26, 32). In our experiments, the *scid* mutation predominantly induced large deletions in DGR that often removed the entire 5′ end of DGR and extended an unknown distance into the flanking genomic DNA (Fig. 1). Thus, rearrangement of DGR faithfully mimicked rearrangements at endogenous loci in *scid* pre-B cells. In one DGR clone, 9-10-1, the deletion was localized specifically to the coding junction, whereas the signal junction had formed normally. On the basis of these data, we speculated that reciprocal fragments in every DGR inversion rearrangement always formed normally but were then subsequently deleted by an inability to repair the coding junctions efficiently. The reciprocal junction of DGR is located only 2.2 kb from the coding junction, and many of the unselected deletions proceeded beyond this position, rendering many of the clones uninformative with regard to this hypothesis. Fortunately, however, this hypothesis could be readily tested by examining endogenous inversional rearrangements in which the signal junctions were separated by many kilobases on the chromosome from the position of the coding junction. Indeed, normal reciprocal fragments, but no functional coding junctions, were obtained when five endogenous inversional light-chain gene rearrangements in *scid* pre-B cells were analyzed (3). Unfortunately, the reciprocity of any two junctions with respect to each other could not be established. We overcame this shortcoming by selecting for MPA resistance to effectively minimize the extent of deletions in DGR. Six DGR inversions were obtained from *scid* cells in which both the reciprocal and coding joints were recovered

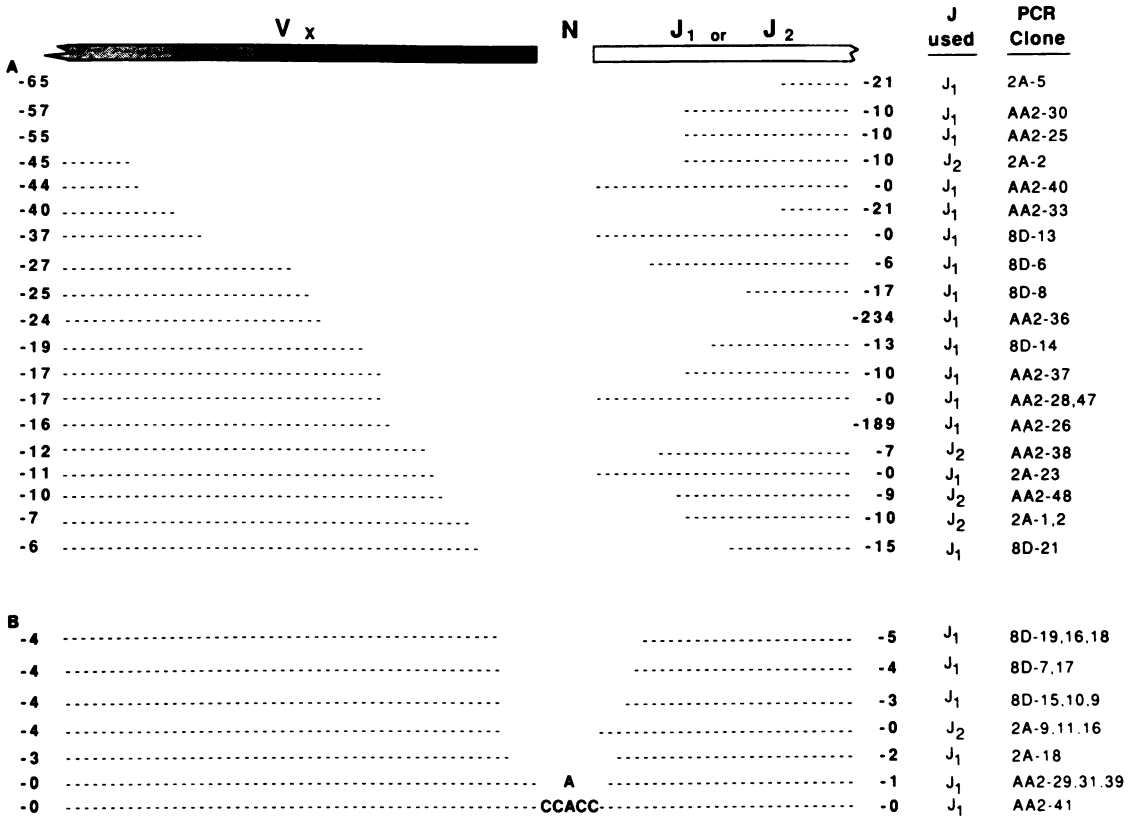

FIG. 7. Detection of wild-type rearrangements in *scid* pre-B cells. DNA sequences of V-to-J coding junctions for light-chain rearrangements were determined from *scid* pre-B cell lines. See Fig. 5 and 6 and Materials and Methods for details. Wild-type rearrangements are grouped by having <6-nt deletions of Vκ and Jκ coding elements.

within the same molecule. Analysis of these inversions clearly showed that coding-joint formation was much more sensitive to the effects of the *scid* mutation than reciprocal fragment formation (Fig. 3). Thus, the *scid* mutation affects lymphoid gene rearrangement in a highly asymmetric fashion: coding joints are preferentially, but not always, affected, whereas formation of reciprocal fragments appears largely unimpaired.

This conclusion is in good agreement with results previously obtained with endogenous recombination substrates (3, 8) and transient transfection of recombination templates (20) introduced into *scid* pre-B cells. Some aspects of the results, however, varied between the two experimental systems. In the transient transfection system, coding joints could not be detected at all and the signal joints formed were unusual in that 50% contained deletions. Data obtained from wild-type cells in the same assays showed deletions in only 0.3% of the reciprocal fragments (20, 21). With endogenous recombination systems, coding junctions could be detected (8; this work) and reciprocal fragments were normally undeleted (3, 8; this work). We have considered several explanations for these experimental variations. First, the drug selection assay for DGR inversions may simply be more sensitive than the drug selection-colony formation assay of Lieber et al. (20). In addition, with the transient transfection system, the formation of the coding and signal joints can be assayed individually, whereas with integrated inversional substrates, the formation of both junctions is required concomitantly. This constraint could bias the results with integrated inversional substrates toward wild-type events. An alternate explanation may be that topological differences exist between chromosomal and extrachromosomal recombination templates and lead to pronounced changes in the ability to reestablish coding and reciprocal junctions. Also, since the copy number of rearrangement templates is very large with the transfection assay system, some limiting factor(s) necessary for coding-joint and reciprocal-fragment formation may be diluted by template excess. We detected proper coding-joint and reciprocal-fragment formation in cells harboring a single DGR substrate.

What is the molecular basis of the *scid* mutation? We have demonstrated that the *scid* gene encodes a *trans*-acting factor by showing that it can aberrantly affect V(D)J recombination on an ectopic rearrangement template (10; this work). These experiments indicate that the *scid* protein serves as a component of the V(D)J recombination mechanism, presumably involved in the joining of the coding junction. Our results are most consistent with the *scid* mutation simply being poorly penetrant. Since measurable wild-type immunoglobulin rearrangements occurred in *scid* pre-B cells, the mutant gene could potentially be altered by only a point mutation, a small insertion, or a small deletion, such that the *scid* protein is still capable of promoting wild-type activity at low levels. In such a scenario, somatic genetic reversion of the mutation might then be expected, especially because of the large number of progenitor cells proliferating in the lifetime of the animal. Because the mutation is recessive, a genetic reversion of either *scid* allele in a progenitor cell would allow normal immunoglobulin and TCR gene rearrangements to occur and produce functional

B- or T cells. Evidence supportive of reversion of the mutation has recently emerged from characterization of TCR gene rearrangements in functional T cells of leaky *scid* mice (6, 27). However, if the *scid* mutation does revert at an appreciable frequency, one might expect to see the entire repertoire of serum immunoglobulin proteins and T-cell subsets. In fact, leaky *scid* mice usually exhibit a very limited number of serum antibody isotypes and express only one to three immunoglobulin light-chain proteins (5).

We also cannot formally exclude the possibility that a null mutation of the *scid* gene has occurred. Consequently, wild-type rearrangement junctions would result from a secondary mechanism that could substitute for the *scid* gene product. It has been suggested that such secondary mechanisms may be essential for repairing aberrant deletions caused by the *scid* mutation (20). However, the presence of wild-type V-Jκ junctions from the same rearrangement that produces normal signal joints is difficult to explain by an alternate illegitimate recombination pathway that would so precisely seal chromosome breaks in the absence of a normal V(D)J joining mechanism. These issues can be fully resolved with the cloning of the *scid* gene.

μ protein is not essential for κ-gene rearrangement. The mechanism of initiation of κ-gene rearrangement is not known, but the primary model has been an induction based on the presence of μ protein (for a review, see reference 2). If a μ signaling model is correct, one would predict that *scid* pre-B cells would fail to rearrange κ genes, since they are almost uniformly defective in assembling endogenous μ proteins. We have shown here that κ-gene rearrangement occurs in *scid* cells with or without LPS stimulation (Fig. 5). All three *scid* pre-B cell lines assayed had defective endogenous heavy-chain rearrangements and were incapable of producing μ protein (10). In addition, we were unable to induce κ-gene rearrangement in these same cell lines after introduction of a rearranged μ gene by electroporation (Hendrickson and Weaver, unpublished results). Recently, Blackwell et al. (3) reported the detection of aberrant κ-gene rearrangements in *scid* pre-B cells that also lacked μ protein. Similarly, when a rearranged μ gene was transfected into these cells, κ-gene rearrangement correlated only with germ line κ transcription but not with μ expression (3). Studies analyzing the activation of κ-gene rearrangement in cell lines incapable of producing μ protein also found a correlation only with the transcriptional induction of the κ-gene J locus (31). Lastly, four human Epstein-Barr virus-immortalized pre-B-cell clones have been described that produce κ light-chain proteins without any detectable μ mRNA or protein (15).

Our results taken together with previous work suggest that a heavy-chain protein-independent pathway for initiating κ-gene rearrangement must exist. However, in the mouse, a correlation of μ synthesis with initiation of κ-gene rearrangement has been produced for one cell line, 300-19, that has the capacity of ongoing rearrangement of light-chain genes in culture (28; reviewed in reference 2). The most likely consensus model is that μ is not the only signal involved in κ-gene rearrangement and perhaps not even a necessary one. Future experiments will be directed toward determining whether μ protein is a primary component in the induction of κ-gene rearrangement during the differentiation of B cells.

ACKNOWLEDGMENTS

We gratefully acknowledge helpful discussions with our colleagues, particularly David Baltimore, Ranjan Sen, Michael Lieber, and Michael Lenardo. We thank Haruo Saito and John Petrini for helpful criticisms and discussions pertaining to the manuscript.

E.A.H. was supported by Public Health Service postdoctoral fellowship 5F32HD07034-02 from the National Institutes of Health. M.S.S. is a Bristol-Myers Cancer Research Fellow. D.T.W. was supported in part by Public Health Service grant GM39312 from the National Institutes of Health.

LITERATURE CITED

1. **Alt, F. W., and D. Baltimore.** 1982. Joining of immunoglobulin heavy chain gene segments: implications from a chromosome with evidence of three D-J_H fusions. Proc. Natl. Acad. Sci. USA **79**:4118–4122.
2. **Blackwell, T. K., and F. W. Alt.** 1988. Immunoglobulin genes, p. 1–60. *In* B. D. Hames and D. M. Glover (ed.), Molecular immunology. IRL Press, Oxford.
3. **Blackwell, T. K., B. A. Malynn, R. R. Pollock, P. Ferrier, L. R. Covey, G. M. Fulop, R. A. Phillips, G. D. Yancopoulos, and F. W. Alt.** 1989. Isolation of *scid* pre-B cells that rearrange kappa light chain genes: formation of normal signal and abnormal coding joins. EMBO J. **8**:735–742.
4. **Bosma, G. C., R. P. Custer, and M. J. Bosma.** 1983. A severe combined immunodeficiency mutation in the mouse. Nature (London) **301**:527–530.
5. **Bosma, G. C., M. Fried, R. P. Custer, A. Carroll, D. M. Gibson, and M. J. Bosma.** 1988. Evidence of functional lymphocytes in some (leaky) *scid* mice. J. Exp. Med. **167**:1016–1033.
6. **Carroll, A. M., and M. J. Bosma.** 1988. Detection and characterization of functional T cells in mice with severe combined immunodeficiency. Eur. J. Immunol. **18**:1965–1971.
7. **Dorschkind, K., G. Keller, R. Phillips, R. Miller, G. Bosma, M. O'Toole, and M. Bosma.** 1984. Functional status of cells from lymphoid and myeloid tissues in mice with severe combined immunodeficiency disease. J. Immunol. **132**:1804–1808.
8. **Ferrier, P., L. R. Covey, S. C. Li, H. Suh, B. A. Malynn, T. K. Blackwell, M. A. Morrow, and F. W. Alt.** 1990. Normal recombination substrate V_H to DJ_H rearrangements in pre-B cell lines from *scid* mice. J. Exp. Med. **171**:1909–1918.
9. **Hanahan, D.** 1985. Techniques for transformation of *E. coli*, Vol. I, p. 109–135. *In* D. M. Glover (ed.), DNA cloning: a practical approach. IRL Press, Oxford.
10. **Hendrickson, E. A., D. G. Schatz, and D. T. Weaver.** 1988. The *scid* gene encodes a *trans*-acting factor that mediates the rejoining event of Ig gene rearrangement. Genes Dev. **2**:817–829.
11. **Hesse, J. E., M. R. Lieber, K. Mizuuchi, and M. Gellert.** 1989 V(D)J recombination: a functional definition of the joining signals. Genes Dev. **3**:1053–1061.
12. **Kabat, E. A., T. T. Wu, M. Reid-Miller, H. M. Perry, and K. S. Gottesman.** 1987. Sequences of proteins of immunological interest, 4th ed. U.S. Department of Health and Human Services, National Institutes of Health, Bethesda, Md.
13. **Kim, M.-G., W. Schuler, M. J. Bosma, and K. B. Marcu.** 1988. Abnormal recombination of *Igh* D and J gene segments in transformed pre-B cells of *scid* mice. J. Immunol. **141**:1341–1347.
14. **Kronenberg, M., G. Siu, L. Hood, and N. Shastri.** 1986. The molecular genetics of the T-cell antigen receptor and T-cell antigen recognition. Annu. Rev. Immunol. **4**:529–591.
15. **Kubagawa, H., M. D. Cooper, A. J. Carroll, and P. D. Burrows.** 1989. Light chain gene expression before heavy-chain gene rearrangement in pre-B cells transformed by Epstein-Barr virus. Proc. Natl. Acad. Sci. USA **86**:2356–2360.
16. **Landau, N., D. Schatz, M. Rosa, and D. Baltimore.** 1987. Increased frequency of N-regional insertion in a murine pre-B cell line infected with a terminal deoxynucleotidyl transferase retroviral expression vector. Mol. Cell. Biol. **7**:3237–3243.
17. **Lewis, S., A. Gifford, and D. Baltimore.** 1984. Joining of V_κ to J_κ gene segments in a retroviral vector introduced into lymphoid cells. Nature (London) **308**:425–428.
18. **Lewis, S., A. Gifford, and D. Baltimore.** 1985. DNA elements are asymmetrically joined during the site-specific recombination of kappa immunoglobulin genes. Science **228**:677–685.
19. **Lewis, S. M., J. E. Hesse, K. Mizuuchi, and M. Gellert.** 1988.

Novel strand exchanges in V(D)J recombination. Cell **55**:1099–1107.

20. **Lieber, M. R., J. E. Hesse, S. Lewis, G. C. Bosma, N. Rosenberg, K. Mizuuchi, M. J. Bosma, and M. Gellert.** 1988. The defect in murine severe combined immune deficiency: joining of signal sequences but not coding segments in V(D)J recombination. Cell **55**:7–16.
21. **Lieber, M. R., J. E. Hesse, K. Mizuuchi, and M. Gellert.** 1988. Lymphoid V(D)J recombination: nucleotide insertion at signal joints as well as coding joints. Proc. Natl. Acad. Sci. USA **85**:8588–8592.
22. **Malynn, B. A., T. K. Blackwell, G. M. Fulop, G. A. Rathburn, A. J. Furley, P. Ferrier, L. B. Heinke, R. A. Phillips, G. D. Yancopoulos, and F. W. Alt.** 1988. The *scid* defect affects the final step of the immunoglobulin VDJ recombinase mechanism. Cell **54**:453–460.
23. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Morzycka-Wroblewska, E., F. E. H. Lee, and S. V. Desiderio.** 1988. Unusual immunoglobulin gene rearrangement leads to replacement of recombinational signal sequences. Science **242**: 261–263.
25. **Nelson, K., D. Kelley, and R. Perry.** 1985. Inducible transcription of the unrearranged κ constant region locus is a common feature of pre-B cells and does not require DNA or protein synthesis. Proc. Natl. Acad. Sci. USA **82**:5305–5309.
26. **Okazaki, K., S.-I. Nishikawa, and H. Sakano.** 1988. Aberrant immunoglobulin gene rearrangement in *scid* mouse bone marrow cells. J. Immunol. **141**:1348–1352.
27. **Petrini, J. H.-J., A. M. Carroll, and M. J. Bosma.** 1990. T-cell receptor gene rearrangements in functional T-cell clones from severe combined immune deficient (*scid*) mice: reversion of the *scid* phenotype in individual lymphocyte progenitors. Proc. Natl. Acad. Sci. USA **87**:3450–3453.
28. **Reth, M., E. Petrac, P. Wiese, L. Lobel, and F. W. Alt.** 1987. Activation of Vκ gene rearrangement in pre-B cells follows the expression of membrane-bound immunoglobulin heavy chains. EMBO J. **6**:3299–3305.
29. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
30. **Schatz, D., and D. Baltimore.** 1988. Stable expression of immunoglobulin gene V(D)J recombinase activity by gene transfer into 3T3 fibroblasts. Cell **53**:107–115.
31. **Schlissel, M. S., and D. Baltimore.** 1989. Activation of immunoglobulin kappa gene rearrangement correlates with induction of germline kappa gene transcription. Cell **58**:1001–1007.
32. **Schuler, W., I. J. Weiler, A. Schuler, R. A. Phillips, N. Rosenberg, T. W. Mak, J. F. Kearney, R. P. Perry, and M. J. Bosma.** 1986. Rearrangement of antigen receptor genes is defective in mice with severe combined immune deficiency. Cell **46**:963–972.
33. **Tonegawa, S.** 1983. Somatic generation of antibody diversity. Nature (London) **302**:575–581.
34. **Vogelstein, B., and R. Gillespie.** 1979. Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA **76**:615–619.