Unexpected Rearrangement and Expression of the Immunoglobulin \(\lambda 1\) Locus in Scid Mice

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Abstract

In severe combined immunodeficient (scid) mice, V(D)J recombination is severely impaired due to a recessive mutation (scid). Thus, we were surprised to find in this study that \(V\lambda 1-J\lambda 1\) rearrangement is routinely detectable in scid fetal liver, adult bone marrow, and spleen in the apparent absence of completed \(VH-DJH\) and \(Vk-Jk\) rearrangements. Particularly surprising, we found the level of \(V\lambda 1-J\lambda 1\) rearrangement in scid fetal liver to be comparable to that in fetal liver of wild-type mice. The majority of scid \(V\lambda 1-J\lambda 1\) rearrangements contained abnormal deletions at the VJ junction, consistent with the known effect of scid. However, \(\sim 15\%\) of \(V\lambda 1-J\lambda 1\) rearrangements lacked abnormal deletions. Productive \(\lambda 1\) transcripts resulting from in-frame rearrangements were readily detectable in scid adult bone marrow and spleen, consistent with our ability to detect \(\lambda 1\)-expressing cells by flow cytometry in the spleens of bcl-2-transgenic scid mice. Strikingly, \(\lambda 1\) transcripts from individual scid mice often showed VJ junctional sequences with the same recurring palindromic (P) additions of three, four, or five nucleotides. To account for these findings, we suggest that (a) nonhomologous end joining of \(V\lambda 1\) and \(J\lambda 1\) coding ends in fetal B lineage cells may not be (severely) impaired by scid; (b) recurring P additions in scid \(\lambda 1\) transcripts may reflect certain molecular constraints imposed by scid on the resolution of \(V\lambda 1\) and \(J\lambda 1\) hairpin coding ends; and (c) scid lymphocytes with productively rearranged \(V\lambda 1\) and \(J\lambda 1\) elements may differentiate into recombinase-inactive cells and emigrate from bone marrow to spleen.

Key words: B cell differentiation • pro-B cells • premature Ig\(\lambda\) recombination • VJ\(\lambda\) junctional diversity • P additions

Introduction

The rearrangement of Ig genes proceeds in an ordered fashion (for review see references 1 and 2). It begins with rearrangement of the H chain gene elements, DH and JH, followed by VH–DJH rearrangement (3). H chain gene rearrangement is initiated at the pro-B cell stage (4–6), with V\(\lambda\)-J\(\lambda\) rearrangement occurring later (7–10) and/or less frequently (11–13) than V\(k\)-J\(k\) rearrangement. The order of H and L chain gene rearrangement is not absolute, however, as studies with B lineage cell lines (14–16) and sorted pro-B cells (5, 6) indicate that rearrangement at the \(k\) or \(\lambda\) locus may precede or occur independently of H chain gene rearrangement. Also, inactivation of the \(k\) locus by gene targeting has shown that rearrangement of the \(k\) locus does not require prior rearrangement of the \(\lambda\) locus (17, 18), consistent with earlier evidence for independent rearrangement of \(\lambda\) and \(k\) loci in various cell lines (16, 19–21) and in \(k\)-transgenic mice (22). Independent rearrangement of \(\lambda\) and \(k\) loci is in agreement with a stochastic model of L chain gene rearrangement (11, 23) and contrary to a strictly regulated model of L chain gene rearrangement (for review see reference 2).

Consistent with the stochastic model of L chain gene rearrangement, we report here a low frequency of \(V\lambda 1-J\lambda 1\) rearrangement in severe combined immunodeficient (scid) and in wild-type (wt)\(^3\) mice with a targeted deletion of the JH locus (JHT mice) (24). As JHT mice lack a functional H chain locus, \(V\lambda 1-J\lambda 1\) rearrangement in these mice must occur independently of H chain rearrangement. Furthermore, as both scid and JHT mice lack pre-B cells, the observed \(V\lambda 1-J\lambda 1\) rearrangement is inferred to occur at the

\(^3\) Abbreviations used in this paper: DNA-PKcs, DNA protein kinase catalytic subunit; JHT, JH targeted deletion; P, palindromic; RAG, recombination activation gene; RT, reverse transcriptase; TdT, terminal deoxynucleotidyl transferase; wt, wild-type.
pro-B cell stage and independently of Vκ-Jκ rearrangement.

Although a low frequency of Vκ1-Jκ1 rearrangement at the pro-B cell stage in JHT mice could have been predicted based on the stochastic model of L chain gene rearrangement, the regular occurrence of such rearrangement in scid pro-B cells would not have been predicted. In scid mice, V(D)J recombination is severely impaired as a result of a DNA repair defect (25–27). The defect is due to a nonsense mutation in the gene coding for the catalytic subunit of DNA protein kinase (DNA-PKcs) (28–30). Because of this mutation, developing scid lymphocytes cannot efficiently join V, D, and J coding ends resulting from the initiation of V(D)J recombination (31–34). Consequently, most developing scid lymphocytes are thought to die prematurely with persisting DNA breaks. Therefore, given the presence of a DNA repair defect (25–27). The defect is due to a nonsense mutation in the gene coding for the catalytic subunit of DNA protein kinase (DNA-PKcs) (28–30). Because of this mutation, developing scid lymphocytes cannot efficiently join V, D, and J coding ends resulting from the initiation of V(D)J recombination (31–34).

Our findings raise several puzzling issues: (a) Why is the level of Vκ1-Jκ1 rearrangement comparable in fetal liver of scid and wt mice; (b) Why do most λ1 transcripts of individual scid mice show the same recurring P additions; and (c) How do λ1 expressing scid cells survive the deleterious effect of scid and apparently emigrate from bone marrow to spleen? These three issues are discussed.

Materials and Methods

Mice. C.B-17 mice homozygous and heterozygous for the scid mutation (41) are here denoted as scid and scid/+ mice, respectively. C.B-17 scid mice hemizygous for the bcl-2-36 transgene (bcl-2 scid mice; reference 42) were obtained from S. Cory (The Walter and Eliza Hall of Medical Research, Melbourne, Australia). Genotyping of bcl-2 mice was done by PCR using DNA from tail snips (43) and oligonucleotide primers for the SV 40 sequence included in the transgene (44). Mice with both of their recombinant activation gene (RAG)1 loci inactivated by gene targeting (RAG−/− mice; reference 45) and with their Jκ elements deleted by gene targeting (24) were provided by R. Hardy (Fox Chase Cancer Center). The targeted JHT allele was backcrossed onto C.B-17 mice for three backcross generations (N 3). N 3F1 mice were intercrossed to generate N 3F2 mice homozygous for the JHT allele (JHT mice). JHT mice were crossed with scid mice to obtain JHT/+, scid/+; these were then intercrossed to obtain JHT scid mice. Genotyping for the wt and inactivated Jκ allele was done by PCR using tail DNA and primers specific for the wt and inactivated Jκ locus (see JH 1 and JHT oligonucleotides below). All of the above mice were bred and maintained at the Fox Chase Cancer Center and were analyzed between the ages of 6 and 12 wk.

Flow Cytometric Analysis. Flow cytometry was used to test for the presence of cells with surface Igκ1 (λκ1)− cells in scid and bcl-2 scid mice. In brief, spleen cells of individual scid, scid+, bcl-2 scid, bcl-2 scid+, and RAG−/− mice were stained with biotin-conjugated anti-CD8 (53.6), allopurinolycin (PharMingen)-conjugated anti-CD45 (B220), and FITC-conjugated anti-λκ1 (R 11-153-FITC; PharMingen) in the manner previously described (46). Cells were analyzed by three-color flow cytometry using a dual laser FACS Plus™ (Becton Dickinson). Binding of biotinylated antibodies was revealed by Texas red conjugated streptavidin (Southern Biotechnology). Dead cells were identified by propidium iodide staining and excluded from analysis. Gates were set to score λκ1− cells based on the distribution of λκ1 staining of spleen cells in the scid/+ positive controls. Due to the paucity of λκ1− cells in scid and bcl-2 scid mice, between 0.5 and 1.0 × 107 spleen cells were analyzed per mouse. Cells were simultaneously stained for the B and T specific markers B220 and CD8, respectively, to ensure that cells scored as λκ1− were indeed B lineage cells (i.e., λκ1− B220− CD8−). Spleen cells from RAG−/− mice served as negative controls for background staining of λκ1.

Oligonucleotides. Oligonucleotides were synthesized by an Applied Biosystems 394 DNA/RNA Synthesizer. Oligonucleotides used as primers for PCR or reverse transcriptions (RT)-PCR were as follows: VH (#91), 5′-GCCGATCCGAGGCATCCGAGTCGGTGGG-3′; DH (#285), 5′-ACTGCTACCTCCTGCAGGAGCAGCTGGG-3′; VH (#361R), 5′-AGATAATCTGTTACCTAAGGCTCTC-3′; μ (#289), 5′-ATGCGATTCTCCTCCTCTCTCTTGGCCCT-3′; Vκ (#68), 5′-GGCTGCAGGACATTGTCGTCGACCCATCTCAGGCT-3′; jκ1 (#236), 5′-GGTGGACAAATTATCCCTTCTTCTTCTAATC-3′; Cκ (#130), 5′-AGTGAACGGATTGTTGTCGCAGATC-3′; jκ2 (#367), 5′-AGTGAACAATTATCCCTTCTTCTTCTAATC-3′; Cκ (#294), 5′-AGAATCCTCGACTCTCCACCATC-3′; jκ1 (#271), 5′-GACCAGCTAAGTGGACAGACCTGGAG-3′; Cκ (#289), 5′-GAGGAAGTGTTAGGGAAACGGG-3′; βκM1 (#229), 5′-GAATGGGGAAGGGCAGGACATCTGAACTG-3′; βκM2 (#230), 5′-TGCTGTACATGTCTCATTCCACCTC-3′; SV400 (#355), 5′-GGAGCTGAGATAGGAGGCGAGAG-3′; SV40 (#356), 5′-GGAGGAGCAACTCTTATTGCTG-3′; JHT1 (#370), 5′-CCTTGGCCAGCTGTCGCTAGC-3′; JHTR (#371), 5′-GGAGGAGCAACTCTTATTGCTG-3′; GκM1 (#367), 5′-GGATGGGGAGACAGGGGAGACAGGG-3′; Jκ− (368), 5′-GGGAACGGGGGGCTTGAATGTCGCTC-3′; Jκ1 (369), 5′-GGAGGCGAGCTCGCTGCGGTGCCTCTG-3′.
an initial denaturation for 4 min at 95°C, with 23 cycles of 1 min
at 94°C, 45 s at 68°C, and 1 min at 72°C, and a final elongation
step for 5 min at 72°C. 1/10 of each PCR reaction (1/20 for β2M
control) was electrophoresed through 1.5% LE agarose (FM C
Bioproducts) in 1× Tris-acetate-EDTA buffer, turboblotted by
alkaline transfer onto maximum strength Nytran Plus membranes
(Schleicher & Schuell) and hybridized in Denhardt’s solution
with the appropriate probes. Radioactive α-32P-dCTP labeling
was done by random priming using the Prime-It II Kit (Strata-
gene). Hybridization probes included pJH6.3 (47), pECκ (11),
and a PCR-amplified and gel-purified (QiaexII; Qiagen) VJ1
gene fragment to score for DH–JH (or VH–DJH), Vκ–Jκ, and
Vλ1-Jλ1 rearrangements, respectively. As a control for the
amount of input DNA, a portion of the nonrearranging
β2M gene was PCR amplified and hybridized to a β2M-specific
probe. Blots were exposed to X-O mat (Eastman Kodak Co.)
autoradiographic film and also to a PhosphorImaging plate for
quantitation by a BAS1000M ac Bio-Imaging Analyzer (Fuji
Photo Film Co.).

RT-PCR. Total RNA from ~5 × 10⁶ bone marrow or
spleen cells was obtained by using R NEasy (Qiagen) as prescribed
by the manufacturer. RNA was eluted into DEPC-treated H₂O
and stored at −73°C. RNA from the equivalent of ~1.5 × 10⁹
bone marrow cells or ~3.0 × 10⁶ spleen cells was used to synthe-
size first strand cDNA using SuperscriptII RT and 100 ng of ran-
dom hexamers (Amersham Pharmacia Biotech) as directed by the
manufacturer (GIBCO BRL). A portion of this cDNA (equiva-
 lent to ~3 × 10⁶ bone marrow cells or ~6 × 10⁶ spleen cells)
was amplified by PCR using 220 μM each of dATP, dGTP,
dCTP, and dTTP, 0.4 μM primers, 20 μM Tris-HCl, pH 8.4,
50 μM KCl, 1.5 μM MgCl₂, and 2.5 U of AmpliTaq DNA poly-
merase (PerkinElmer) in a reaction volume of 50 μl. Controls for
nonspecific amplification of PCR products included RAG−/−
cDNA and no cDNA template in the reaction. Semiquantitative
PCR using a PTC-100 Thermal Controller (MJ Research) was
carried out after an initial denaturation for 4 min at 95°C, with 23
cycles of 1 min at 94°C, 45 s at 65°C, and 1 min at 72°C, and a
final elongation step for 5 min at 72°C. Southern blotting and
hybridization was carried out as described above. The hybridiza-
tion probes included pC)m3741 (48), pECκ, and gel-purified
VJ1 and β2M PCR-amplified gene fragments.

Quantitation. Conditions for semiquantitative PCR were de-
termined by varying cycle number and the amount of input
DNA (or cDNA). Filters were exposed to a Fuji imaging plate to
quantify the amount of α-32P-hybridized probe in experimental
samples relative to that in control (reference) samples using a
BAS1000M ac Bio-Imaging Analyzer (Fuji Photo Film Co.). We
found that with 23 cycles of amplification, the amount of PCR
product was proportional to the amount of input DNA (from 10⁶
cells) at several different dilutions. Similarly, at 23 cycles, the
amount of RT-PCR product was found to be proportional to
the amount of input cDNA (from 3–6 × 10⁶ cells) at several dif-
ferent dilutions.

Sequence Analysis. To ensure sufficient PCR product for
cloning, one microliter from the primary PCR or RT-PCR re-
action was subjected to an additional 15 cycles of PCR using
conditions as above. The Vλ1μ50 primer was used with Jλ1 or Cλ1
for recovery of junctional sequences from genomic DNA or
cDNA, respectively. PCR products were electrophoresed
through 1.5% LE agarose, purified using QiaexII, and cloned into
cPCR2.1 for transformation of IN3C(+) bacteria (Invitrogen). Re-
combinant colonies were randomly chosen for plasmid recovery
by Perfect Prep (SPrime-3Prime, Inc.). Plasmids were submitted
for cycle sequencing using the ABI Prism Dye Terminator Reac-
tion Kit and an ABI 377 DNA Sequencer (PerkinElmer).

Results

Evidence for Vλ1-Jλ1 Rearrangement before the Pre-B cell Stage. To test whether the λ1 locus can rearrange early in
B cell differentiation, we assayed for the presence of non-
germline λ1 transcripts in scid mice and also in bcl-2 scid
mice. As shown in Fig. 1 A, λ1 transcripts resulting from
Vλ1-Jλ1 rearrangement (λ1 transcripts) were clearly evi-
dent in the bone marrow of scid mice and more so in the
bone marrow of bcl-2 scid mice. The higher abundance of
λ1 transcripts in the latter mice presumably reflects the
greater longevity of B lineage cells in bcl-2 scid mice than
in scid mice (42). Fig. 1 A also illustrates that transcripts re-

Figure 1. (A) Detection of λ1 transcripts in bone marrow of individual
scid (s/s) and bcl-2 s/s mice and (B) in pooled bone marrow of JHT s/s
and JHT non-scid (+/+ ) mice. Transcripts were detected by RT-PCR
using locus-specific primers (see Materials and Methods). Transcripts cor-
responding to DH–JH, VH–DJH, Vκ–Jκ, and Vλ1-Jλ1 rearrangements
are denoted Dμ, μ, κ, and λ1, respectively. Amplification of β2M tran-
scripts served as a control for the amount of input cDNA. Results ob-
tained with wt (+/+ ) bone marrow are provided for comparison.
sulting from \( V\kappa-J\kappa \) rearrangement (\( k \) transcripts) were routinely detectable in bone marrow of bcl-2 scid but not scid mice, whereas transcripts resulting from \( VH-DJH \) rearrangement (\( \mu \) transcripts) were barely detectable in some bcl-2 scid mice and not at all in scid mice (Fig. 1 A). Consistent with previous reports of detectable DH–JH rearrangement in scid mice (49, 50), DH–JH transcripts resulting from DH–JH rearrangement were readily detectable in scid and bcl-2 scid bone marrow.

As differentiation of scid B lineage cells does not generally progress beyond the pro-B cell stage, our detection of \( \lambda1 \) transcripts in scid mice suggests that \( V\lambda1-J\lambda1 \) rearrangement may occur before the pre-B cell stage. To test whether this is indeed true and whether the \( \lambda1 \) locus can rearrange independently of the H chain locus, we assayed transcripts in bone marrow of JHT mice. In these mice, B cell differentiation is completely arrested at the pre-B cell stage as a result of gene-targeted inactivation of the bcl-2 locus (24). Fig. 1 B shows that \( \lambda1 \) transcripts were readily detectable in JHT bone marrow. The abundance of \( \lambda1 \) transcripts in JHT mice was ~40-fold less than in wt mice but ~25-fold greater than in JHT scid mice (Table I). We conclude that \( V\lambda1-J\lambda1 \) rearrangement can occur at the pro-B cell stage and independently of the H chain gene rearrangement.

Developmental onset of \( V\lambda1-J\lambda1 \) rearrangement in scid and wt embryos. To compare the developmental onset of \( V\lambda1-J\lambda1 \) rearrangement with that at other Ig loci, we tested genomic DNA from pooled livers of scid and wt embryos for DH–JH, VH–DJH, \( V\kappa-J\kappa \), and \( V\lambda1-J\lambda1 \) rearrangement (Fig. 2). In wt embryos, we found that DH–JH rearrangement could be detected as early as day 12, whereas VH–DJH and \( V\kappa-J\kappa \) rearrangements were not detectable until day 14. These results are in general agreement with earlier reports on the time course of H and \( \kappa \) chain gene rearrangement in fetal mice (51, 52). In scid embryos, DH–JH rearrangement was not clearly evident until day 14, and VH–DJH and \( V\kappa-J\kappa \) rearrangements were not detected except for a VH–DJH rearrangement of aberrant size in the day 15 sample. In contrast, \( V\lambda1-J\lambda1 \) rearrangement was evidently as early as day 12 in both scid and wt embryos. These results indicate that \( V\lambda1-J\lambda1 \) rearrangement is initiated early in development and may developmentally precede VH–DJH and \( V\kappa-J\kappa \) rearrangement.

It is important to note in Fig. 2, A and B, that the level of \( V\lambda1-J\lambda1 \) rearrangement and \( \lambda1 \) transcripts in day 13–15 scid fetal liver remains relatively constant and appears comparable to that in the day 13 and 14 wt fetal liver. Indeed, quantitation of the amount of \( \lambda1 \) hybridizing signal for \( V\lambda1-J\lambda1 \) rearrangement and \( \lambda1 \) transcript in the day 13 and 14 scid samples showed this to be ~80% of that in the corresponding wt samples (Table I). It should be noted that the observed level of \( V\lambda1-J\lambda1 \) rearrangement in DNA from 10^6 fetal liver cells was about two orders of magnitude

| Genotype | Fetal Liver | Bone Marrow |
|----------|-------------|-------------|
| +/-      | 1.0         | 1.0         |
| s/s      | 1.2, 0.52   | -           |
|          | (0.76, 0.83)| -           |
| JHT s/s  | -           | 0.001       |
| JHT +/+  | -           | 0.024       |

PCR-amplified (23 cycles) \( \lambda1 \) transcripts, and also \( V\lambda1-J\lambda1 \) rearrangements in the case of fetal liver, were gel electrophoresed, blotted, and hybridized with a \( \lambda1 \)-specific probe (see Materials and Methods). The values shown correspond to the amount of \( \lambda1 \) hybridizing signal normalized against the internal control (\( \beta2M \)) and the reference control, fetal liver or bone marrow of wt (+/-) mice. Thus, for example, the ratio of \( \lambda1/\beta2M \) hybridizing signal in JHT (JHT +/-) bone marrow divided by the \( \lambda1/\beta2M \) hybridizing signal in the reference control equated 0.024. Two values are shown for scid fetal liver. These correspond to the ratios obtained for day 13 and 14 samples, respectively. The two values in parentheses correspond to the ratios obtained for \( V\lambda1-J\lambda1 \) rearrangement in genomic DNA from day 13 and 14 scid fetal liver, respectively.

![Figure 2](image.png)
less than in control DNA samples from 10^6 adult bone marrow cells of wt mice (data not shown). This is not surprising, as day 13–14 fetal liver lacks detectable pre-B cells and contains <1% pre-B cells (reference 53 and our unpublished results).

\[ V_{\lambda 1} - J_{\lambda 1} \] Rearrangements from scid Mice Contain Abnormal Deletions at the VJ Junction. PCR-amplified \( V_{\lambda 1} - J_{\lambda 1} \) rearrangements were detectable not only in scid fetal liver and adult bone marrow, but also in scid adult spleen. In most of these rearrangements, the \( V_{\lambda 1} \) and/or \( J_{\lambda 1} \) coding segments were abnormally truncated by >20 nucleotides. Deletions of this magnitude were not observed in \( V_{\lambda 1} - J_{\lambda 1} \) rearrangements from wt mice. Representative results are illustrated in Figs. 3 and 4 for adult bone marrow and spleen.

Figure 3. Representative \( V_{\lambda 1} - J_{\lambda 1} \) junctional sequences in genomic DNA from pooled fetal liver (FL) of scid (s/s) embryos (day 13) and from pooled bone marrow (BM) of s/s, wt (+/1), and JHT adult mice. Germ-line nucleotides (nt) for the 3’d and 5’d ends of the \( V_{\lambda 1} \) and \( J_{\lambda 1} \) gene are shown at the top (the germline \( V_{\lambda 1} \) and \( J_{\lambda 1} \) coding regions comprise 297 and 38 nt, respectively). Upper- and lowercase letters under the N/P column denote N and P nucleotide additions, respectively. The number of V or J nucleotides deleted from the \( V_{\lambda 1} \) or \( J_{\lambda 1} \) coding end is indicated in parentheses; the asterisk denotes an in-frame rearrangement.

Figure 4. Representative \( V_{\lambda 1} - J_{\lambda 1} \) junctional sequences in genomic DNA of pooled spleen (SPL) from scid (s/s) and wt (+/+) adult mice. Format is as in Fig. 3.

More than 70% of scid \( V_{\lambda 1} - J_{\lambda 1} \) rearrangements (121/147 distinct sequences analyzed) contained abnormal deletions; those lacking such deletions often showed unusually long P additions, as illustrated in Fig. 4.

\( V_{\lambda 1} - J_{\lambda 1} \) rearrangements from bone marrow of JHT mice, in contrast to those from bone marrow of scid mice, showed nontemplated (N) additions and comparatively small deletions at the VJ junction (Fig. 3). N addition is dependent on terminal deoxynucleotidyl transferase (TdT) (54, 55). This enzyme is expressed at the pro-B cell stage (56), the stage at which B cell differentiation is arrested in JHT mice (24). The absence of N additions in \( V_{\lambda 1} - J_{\lambda 1} \) junctions from wt mice (Figs. 3 and 4) is in agreement with earlier reports (57, 58) and consistent with the occurrence of most L chain rearrangement at the late pre-B cell stage (5, 6), when TdT expression is dramatically downregulated (56). As scid and JHT mice both show an arrest of B cell differentiation at the pro-B stage, the abnormal loss of nucleotides in scid \( V_{\lambda 1} - J_{\lambda 1} \) junctions must reflect the effect of the scid mutation and not a peculiarity of premature \( V_{\lambda 1} - J_{\lambda 1} \) rearrangement.

\( \lambda 1 \) Transcripts in scid Adult Bone Marrow and Spleen Lack Abnormal Deletions at Their VJ Junction. Most \( \lambda 1 \) transcripts from scid adult bone marrow and spleen corresponded to in-frame \( V_{\lambda 1} - J_{\lambda 1} \) rearrangements with frequent P additions (illustrated in Fig. 5). The P additions consisted of three to five nucleotides (cag, ccag, and gaat) and were found repeatedly in individual mice. The most common recurring sequence consisted of two P additions separated by an AT dinucleotide (gaat-AT-ccag). Interestingly, the AT dinucleotide is palindromic to the last two nucleotides of the (gaat) P addition and were found repeatedly in individual mice. The most common recurring sequence consisted of two P additions separated by an AT dinucleotide (gaat-AT-ccag). Interestingly, the AT dinucleotide is palindromic to the last two nucleotides of the (gaat) P addition. Similar restricted VJ junctional sequences and recurring P additions were also observed in \( \lambda 1 \) transcripts recovered from bcl-2 scid spleen (data not shown). It is important to note that each PCR amplification of bone marrow and splenic cDNA from scid mice was done in parallel with PCR amplification of splenic cDNA from wt and RAG1^-/- mice. We found
that \( \lambda_1 \) transcripts from three individual wt mice lacked N/P additions (illustrated in Fig. 5). No \( \lambda_1 \) transcripts were recovered from cDNA of RAG1-2 mice. Thus, the observed recurring P additions appear to be a unique property of \( \lambda_1 \) transcripts in the bone marrow and spleens of scid mice.

We analyzed a total of 188 cloned sequences from \( \lambda_1 \) transcripts in bone marrow and/or spleens of five individual scid mice. We found that 115 clones contained P additions. In Fig. 6, each distinct junctional sequence (denoted A–I) among the 115 clones is listed according to its representation in individual mice and overall frequency (see histogram). Sequences B and C are treated as one in the histogram, as are sequences F and G, because each of these pairs is identical except for the substitution of T (underlined) for C in the \( V_L \) germline codon, AGC. Note that (a) sequences A–G, comprising most of the clones (110/115), corresponded to in-frame \( V_L-J_L \) rearrangements; (b) sequences E, F, and G accounted for \(~70\%\) (83/115) of the \( V_L-J_L \) rearrangements and (c), the gaat-AT-cag P addition was present in all mice analyzed and represented nearly 40\% of the clones (45/115).

In contrast to scid mice, \( \lambda_1 \) transcripts from JHT scid mice lacked P additions and showed abnormal deletions at their VJ junctions similar to scid genomic \( V_L-J_L \) rearrangements; furthermore, most corresponded to out-of-frame \( V_L-J_L \) rearrangements (illustrated in Fig. 5). These results suggest, as discussed later, that survival of \( \lambda_1 \)-expressing scid cells could depend on the coexpression of a D\(_M\) (or \( \mu \)) chain. Consistent with this possibility, D\(_M\) and \( \lambda_1 \) transcripts were the only Ig gene transcripts routinely detectable in scid bone marrow (illustrated in Fig. 1). Moreover, most scid D\(_M\) transcripts (10/16 analyzed) corresponded to DH–JH rearrangements in reading frame 2 (data not shown), which would be expected to result in the expression of a D\(_M\) chain.

Cell Surface Expression of \( \lambda_1 \) Chains Is Detectable in Spleens of bcl-2 scid Mice. The presence of \( \lambda_1 \)-expressing scid cells in the spleen prompted us to test for possible cell surface expression of \( \lambda_1 \) chains (\( \lambda_1 \) cells) in the spleens of scid and bcl-2 scid mice. The latter mice were included because survival of scid B lineage cells is known to be enhanced in the presence of the bcl-2 transgene (42). Large numbers (5–10\(^3\)) of cells were analyzed by three-color flow cytometry for expression of cell surface \( \lambda_1 \) and the B and T cell markers CD45 (B220) and CD8, respectively. Mice with an inactivated RAG1 locus (RAG1-2 mice; reference 45) served as a negative control for background staining of \( \lambda_1 \). As illustrated in Fig. 7, there were no detectable \( \lambda_1^+ \)B220+CD8- cells in scid and RAG1-2 mice. However, in bcl-2 scid mice, \( \lambda_1^+ \)B220+CD8- cells were detectable at a frequency of 0.02–0.05% versus 0.5–1.5% in

**Figure 5.** Representative VJ junctional sequences in \( \lambda_1 \) transcripts from individual scid (s/s), wt (+/+), and JHT scid mice. Both bone marrow (BM) and spleen (SPL) of three s/s and three +/+ mice was analyzed; the results for two individuals are shown. Format is as in Fig. 3.

**Figure 6.** Recurring P additions in VJ junctions of \( \lambda_1 \) transcripts from scid adult mice. Bone marrow and/or spleens of five individual scid mice were analyzed. We obtained VJ junctional sequences from 188 clones and found that 115 of these contained P additions. 9 distinct VJ junctional sequences were found among the 115 clones; these are denoted (A–I) below the underlined germline sequence for the \( V_L \) and \( J_L \) coding ends. The number of mice that contained a given VJ junctional sequence is indicated at left. The asterisk denotes that the rearrangements were in-frame. The overall frequency of each distinct junctional sequence (A–I) is shown in the histogram.
the wt controls. The distribution of B220 staining for λ1
gated cells is shown in the histograms on the right side of
Fig. 7. Note that bcl-2 scid spleen cells stained less bright
for B220 and lam than scid/+ or RAG−/− mice, respectively. λ1+ cells in bcl-2 scid mice were based on the distribution of λ1 staining in positive and negative controls, bcl-2 scid/+ and RAG−/− mice, respectively. λ1+ cells in bcl-2 scid/+ and scid/+ control mice are denoted in the shaded areas of the histograms for relative cell number versus λ1 staining. The λ1+ cells in the shaded areas were analyzed for CD8 and B220 expression; most of these cells (~85%) displayed a B cell phenotype (λ1−B220+CD8+) and fell within the boxed areas. The λ1+ cells in bcl-2 scid mice represented ~0.02–0.05% of the spleen cells analyzed and showed a λ1+B220+ phenotype. In contrast, λ1+ cells in scid/+ and bcl-2 scid/+ mice were λ1+B220+ and represented ~0.5–1.5% of the spleen cells analyzed. The overlay of B220 histograms on the far right shows the distribution of B220 staining for cells in the scid/+ and RAG−/− mice and for λ1+ cells in the bcl-2 scid/+ mice.

**Figure 7.** Detection of λ1-expressing cells in spleens of bcl-2 scid (s/s) mice by three-color flow cytometry. Gate settings for scoring λ1+ cells in bcl-2 s/s mice were based on the distribution of λ1 staining in positive and negative controls, bcl-2 s/s and RAG−/− mice, respectively. λ1+ cells in bcl-2 s/s and s/+ control mice are denoted in the shaded areas of the histograms for relative cell number versus λ1 staining. The λ1+ cells in the shaded areas were analyzed for CD8 and B220 expression; most of these cells (~85%) displayed a B cell phenotype (λ1+B220+CD8+) and fell within the boxed areas. The λ1+ cells in bcl-2 s/s mice represented ~0.02–0.05% of the spleen cells analyzed and showed a λ1+B220+ phenotype. In contrast, λ1+ cells in s/+ and bcl-2 s/+ mice were λ1+B220+ and represented ~0.5–1.5% of the spleen cells analyzed. The overlay of B220 histograms on the far right shows the distribution of B220 staining for cells in the s/+ and RAG−/− mice and for λ1+ cells in the bcl-2 s/s, s/+ and bcl-2 s/+ mice.

**Discussion**

The preceding results support earlier evidence, cited in
the Introduction, that initiation of rearrangement at the λ
locus does not require prior rearrangement at the H or κ
chain locus. Our detection of λ1 transcripts in bone mar-
row of scid and JHT mice indicates that Vλ1-Jλ1 rearran-
gement can occur before the pre-B cell stage and inde-
dependently of H chain gene rearrangement. Moreover, the
detection of Vλ1-Jλ1 rearrangement as early as day 12 in
wt fetal liver, in which there is no genetic impairment of Ig
gene rearrangement, suggests that the onset of Vλ1-Jλ1 re-
arrrangement may developmentally precede VH-DJH and
Vκ-Jκ rearrangement. The latter rearrangements were not
detectable before day 14.

The most novel aspect of our findings is the regular de-
tection of Vλ1-Jλ1 rearrangement in scid mice. This
would not have been predicted, particularly the compara-
tive level of Vλ1-Jλ1 rearrangement in scid and wt fetal
liver. Also unexpected are the recurring P additions in scid
λ1 transcripts and the presence of scid cells with in-frame


initiation of Vκ–Jκ rearrangement in scid cells lacking the bcl-2 transgene would be expected to result in persisting chromosomal breaks and cell death.

V(J)Junctions of scid λ1 Transcripts Show Recurring P Additions. Most λ1 transcripts recovered from scid adult mice corresponded to in-frame Vλ1-Jλ1 rearrangements, and >60% of these transcripts contained recurring P additions. Whereas in wt mice most P additions are one or two nucleotides (61), in scid mice they are often longer (62, 63). In the present case, all of the scid P additions at Vλ1 were five nucleotides in length (gaaat), and those at Jλ1 were either three or four nucleotides in length (cag or ccag). This may reflect a strong bias in the resolution of hairpin coding ends imposed by the scid DNA-PKcs deficiency, such that Jλ1 and Vλ1 coding ends are frequently nicked three to four and five nucleotides from the hairpin tip, respectively, and then joined without further modification. In addition, cells expressing λ1 transcripts with these junctions may be strongly selected, as discussed later.

Of particular interest is the recurring Vλ1-Jλ1 junctional sequence of two P additions separated by an AT dinucleotide (gaaat-AT-ccag), which was present in ~40% of the λ1 transcripts with P additions (Fig. 6). The basis for the AT dinucleotide is unclear. Given that scid Vλ1-Jλ1 rearrangements occur at the pro-B cell stage in the presence of high TdT levels, the AT dinucleotide could represent a non-templated addition mediated by TdT. Another possibility is that the AT dinucleotide, which is palindromic to the last two nucleotides of the gaaat P addition, corresponds to a secondary P addition. In this scenario, one could postulate two successive recombination events. The generation of the gaaat P addition would result from an open and shut recombination (64) at Vλ1. This would be followed by secondary cleavage at the Vλ1 signal/coding border, asymmetric nicking of the Vλ1 hairpin coding end, and joining to a Jλ1 coding end with a ccag overhang. Regardless of how the AT dinucleotide is generated, we suggest that cells with the gaaat-AT-ccag junctional sequence are strongly selected to account for the repeated occurrence of this sequence in λ1 transcripts of individual scid mice.

Evidence for λ1-Expressing scid Cells. Scid λ1 transcripts were not only detected in bone marrow but also in spleen. Moreover, λ1 transcripts with the same VJ junctional sequences were often found to recur in both of these tissues. This implies a strong selection for cells with in-frame Vλ1-Jλ1 rearrangements containing particular VJ junctional sequences. Such selection was not evident in JHT scid mice. λ1 transcripts from JHT scid bone marrow contained abnormal deletions at the VJ junction, lacked P additions, and in most cases corresponded to out-of-frame Vλ1-Jλ1 rearrangements. These findings suggest that survival and selection of λ1-expressing cells requires a functional H chain locus.

Vλ1-Jλ1 Transcripts may be in-frame Vλ1-Jλ1 rearrangements in scid bone marrow, pair and associate with the functional H chain locus. One possibility is that Dμ and λ1 chains, resulting from expression of in-frame Dμ and λ1 transcripts in scid bone marrow, pair and associate with the Ig α and β signal-transducing chains (65, 66) to form a B cell.
cell-like receptor (BCR Dμ/λ1). Although Dμ chains cannot pair efficiently with κ chains (67), they can pair with the surrogate L chain (68, 69) and might be expected to pair with λ1 chains as well because the latter share some homology with the surrogate L chain (for review see reference 70). Expression of BCR Dμ/λ1 in this proposed scenario would signal rapid (or direct) progression of scid pro-B cells to the recombinase-inactive B cell stage and allow these cells to survive and migrate to the periphery. But as BCR Dμ/λ1 would lack a VH region, we would not expect cells bearing this receptor to persist or expand in response to naturally occurring antigens. This could in part explain the very low frequency of cells with surface λ1 in bcl-2 scid spleen (≈0.05% of the cells examined). What is experimentally missing in support of the above scenario, however, is evidence for bcl-2 scid cells with surface λ1 and μ chains. Despite reported evidence for intracellular expression of μ (or Dμ) chains in B220+CD22- spleen cells of bcl-2 scid mice (59), cells with surface μ chains have not been detected in bcl-2 scid mice (42, 59).

In conclusion, we suggest that (a) joining of Vα1 and Jα1 coding ends after the initiation of VH1-Jα1 rearrangement may not be impaired in B lineage cells of scid fetal liver because of the developmental time at which such rearrangement occurs; (b) recurring P additions in scid α1 transcripts may reflect a strong bias in the resolution of VH1 and Jα1 hairpin coding ends imposed by the scid defect as well as possible strong selection for cells expressing these transcripts; and (c) pro-B cells with in-frame Vα1-Jα1 rearrangements may express a pre-BCR-like receptor and differentiate into recombinase-inactive cells and emigrate from bone marrow to spleen.

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