V(D)J Recombination in Peritoneal B Cells of Leaky scid Mice

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Summary

Developing lymphocytes in immune-deficient severe combined immunodeficient (scid) mice express a defective recombinase activity and rarely succeed in making an antigen receptor; those cells that do succeed account for the known B and T cell leakiness in this mutant mouse strain. To gain more insight into the nature of the *scid* defect, we assessed the status of heavy (H) and light ($L)_\kappa$ chain genes in immunoglobulin (Ig)M_κ-secreting B cells from the peritoneal cavity of old leaky scid mice, the only lymphoid site where scid B cells have been routinely detected. We found these cells to be unusual in that their nonexpressed H chain alleles were either abnormally rearranged or in germline configuration (wild-type B cells generally show normal rearrangements at both H chain alleles). The VDJ_H junctions of the expressed alleles showed little or no nontemplated (N) addition, similar to neonatal B cells from wild-type mice. About half of the V(D)J junctions lacking N additions contained nucleotides that could have been encoded by either of the participating coding elements (VD_H, DJ_H, or VJ_κ), indicating that the recombination occurred between short stretches of homology. Unusually long templated (P) additions were seen in both VDJ_H and VJ_κ junctions, and many recombinations appeared to involve P-based homologies. These findings suggest that: (*a*) B cell leakiness results from a low frequency of coding joint formation in cells expressing the defective scid recombinase activity; (*b*) joining of scid coding ends is facilitated when the ends contain short stretches of sequence homology, where in many cases, one of the homologous sequences results from a P addition; and (*c*) scid peritoneal B cells may arise early in ontogeny.

In developing lymphocytes, separate and distinct gene elements (V, D, and J) recombine to form VDJ and VJ coding sequences for Ig and TCR V regions (reviewed in reference 1). V(D)J recombination is mediated by a site-specific recombinase that recognizes short DNA sequences (signals) adjacent to each coding element (2–4). The recombination of any two coding elements (e.g., V and J) is thought to involve double strand breaks at the coding/signal borders of both elements followed by rejoining of the DNA ends in a new configuration; i.e., coding to coding and signal to signal (5–8).

In immature lymphocytes of the scid mouse mutant, V(D)J recombination is defective (9–12) and coding joint formation appears to be greatly reduced relative to signal joint formation (10, 12, 13). The basis for this reduction is not yet understood. Recent evidence (14) suggests that coding (but not signal) ends are covalently sealed into hairpin structures before their modification and joining, and that the *scid* mutation impairs the resolution (or increases the production) of hairpins. Impaired resolution of hairpins could result in a high frequency of persisting DNA breaks and cause most scid lymphoid cells to die prematurely.

Some developing scid lymphocytes succeed in forming VDJ and VJ coding joints at two critical antigen receptor loci (e.g., H/L_κ or TCR_β/TCR_α), and given the appropriate stimuli, may expand into a clone of functional B or T cells. Oligoclonal B and T cells have been detected in a low percentage of young adult scid mice (5–20%) and in virtually all old scid mice ($\geq$1 yr old) (15, 16). Two explanations for this B and T cell leakiness have been proposed (15). Leakiness could in part reflect a low rate of somatic reversion (15, 17, 18). Reverted cells, by definition, would express wild-type recombinase activity, and V(D)J rearrangements at both expressed and nonexpressed loci would be normal. Leakiness could also reflect a low frequency of coding joint formation in recombinase-defective scid cells (12, 18–20). In this case, the nonexpressed antigen receptor genes would be expected to show abnormal rearrangements (15, 17) similar to those seen in transformed scid lymphocytes (9), or to remain in germline configuration. The latter expectation is based on the assumption that most attempted scid rearrangements result in cell death, and that survival of developing scid lymphocytes depends on their making productive V(D)J rearrangements with a minimal number of attempts.

To test for evidence consistent with either (or both) of the above explanations, we recovered IgM-producing B cell hy-

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bridomas from the peritoneal cavity of old scid mice, and then analyzed the Ig genes in these hybridomas. We found no clear evidence of reversion in scid peritoneal B cells; rather, these cells exhibited a phenotype consistent with that predicted for recombinase-defective cells. Nonexpressed H chain genes remained in germline configuration or contained abnormal rearrangements similar to those of transformed scid pre-B cells (9). An unexpected feature of scid V(D)J junctions was the low frequency of N addition and high frequency of recombination between short regions of homology. As discussed, this suggests that most peritoneal scid B cells may arise early in ontogeny, and that joining of coding ends in these cells is facilitated when both ends contain short regions of homology.

Materials and Methods

Mice. All mice used in this study were retired, pedigreed foundation breeders from specific pathogen-free colonies of BALB/cAnICR and BALB/cAnICR scid mice in the Fox Chase Cancer Center. The BALB/cAnICR strain is the H chain allotype congenic partner of C.B-17, the strain in which the *scid* mutation spontaneously occurred (21). *scid* was selectively crossed onto the BALB/cAnICR strain by G. Bosma (Fox Chase Cancer Center). BALBc/cAnICR mice homozygous for *scid* are denoted simply as scid mice, and the controls, BALB/cAnICR, as wild-type mice. Both scid and wild-type mice were maintained in microisolator cages (Lab Products, Maywood, NJ) containing sterilized water and food, and were transferred weekly into clean sterile cages within a class II–type safety cabinet (Bellco Glass Inc., Vineland, NJ). The age range of the mice used was from 11 to 24 mo.

FACS® Analysis. Cells from the peritoneal cavity were stained with monoclonal anti-CD45R/B220 (RA3-6B2) conjugated with allophycocyanin and with monoclonal anti-IgM (331.12) conjugated to PE (kindly provided by Dr. R. Hardy, Fox Chase Cancer Center) and analyzed on a dual laser FACStar Plus® (Becton Dickinson & Co., Mountain View, CA). Reagents and staining procedures were as described by Hardy (22). Dead cells were excluded by staining with propidium iodide (1 μg/ml). 30,000 cells were analyzed per sample.

Peritoneal B Cell Hybridomas. Peritoneal cavity cells were cultured with mitogen for 2 d before fusion. Media contained RPMI 1640 (Gibco Laboratories, Grand Island, NY), 15% FCS, 5×10^{-5} M 2-ME, 2 mM L-glutamine, 50 μg/ml gentamycin sulfate, and 10 μg/ml of *Salmonella typhimurium* mitogen, (RIBI, Hamilton, MT). Mitogen-stimulated cells (10^6 cells/ml in 24-well plates) were fused with SP2/0 cells (23) at a ratio of 1:5 using 33% polyethylene Glycol 1000 (Baker, Philipsburg, NJ). The fusion medium was OPTI-MEM I (Gibco Laboratories) with 10% FCS, 5×10^{-5} M 2-ME, and 50 μg/ml gentamycin sulfate. Postfusion medium was as above, supplemented with hypoxanthine-aminopterin-thymidine (HAT), 150 μg/ml oxaloacetate, 50 μg/ml sodium pyruvate, 0.2 U/ml insulin, and 10% Origen hybridoma cloning factor (IGEN, Inc., Rockville, MD).

Protein Dot Blot Assay for IgM-producing Hybridomas. Hybridoma supernatants (1 μl) were spotted onto nitrocellulose filters. The filters were treated with 50% methanol for 30 min, followed by 2.5% casein for 1–2 h. Filters were overlayed sequentially with affinity-purified goat anti-mouse IgM (Fisher Biotech, Malvern, PA) and ^{125}I-IgM (from MOPC 104E), washed in 0.5% casein, and exposed to XAR-5 OMAT film (Kodak). IgM-secreting hybridomas were subcloned by limiting dilution or by plating at one cell/well with a FACStar Plus® (Becton Dickinson & Co.).

Southern Blot Analysis. DNA samples from peritoneal cavity cells of individual leaky scid mice and from IgM-secreting hybridoma clones of scid and wild-type mice were digested with EcoRI, subjected to electrophoresis through 0.7% agarose, and blotted as described previously (9). Blots were hybridized with a J_H-specific probe (pJ11) (24), or with a cDNA probe ($pC_\mu 3741$) specific for IgM C region exons (24). Other blots, containing DNA digested with BamHI or HindIII, were probed with pEC_κ (25) to assess the status of the L_κ locus. Probes were α[^{32}P]dCTP labeled using a nick translation kit (Bethesda Research Laboratories, Gaithersburg, MD).

cDNA Amplification and Cloning. cDNA from the cloned IgM-producing hybridomas was used for PCR amplification of the expressed H and L_κ chain genes (plus the two rearranged, but nonexpressed L_κ chain genes in scid hybridomas Spc4.3 and Spc6.3). The procedures for obtaining the cDNA and for amplifying, cloning, and sequencing the desired gene products were as described previously (18). The PCR primers were synthesized on a DNA synthesizer (381A; Applied Biosystems, Foster City, CA) and are: V_H FR1 (5′-GCCGGATCCGTGCAGCTGGTGGAGTCTGG-3′); C_μ (5′-GCCGGATCCGAGGGGGAAGACATTTGGGAAGGAC-3′); V_κ FR1 (5′-GGCTGCAGGACATTCAGCTGACCCAGTCTCCA-3′); C_κ (5′-ATGGATCCAGTTGGTGCAGCATC-3′).

Cloning and Sequencing of the Nonexpressed Rearranged H Chain Alleles in scid Hybridomas Spc4.3 and Spc6.3. Using forward (V_H FR1, D_{SP}, D_{FL}, or D_{Q52}) and reverse (J_{H2}, J_{H3}, or J_{H4}) primers in different combinations, we attempted to PCR amplify the nonexpressed rearranged H chain alleles from the DNA of scid hybridomas Spc4.3 and Spc6.3. The DNA was prepared using GeneReleaser (BioVentures, Murfreesboro, TN). This approach proved successful for hybridoma Spc6.3 using the following Dsp and J_{H4} nested primers: D_{SP}^{ext}, 290bp 5′ Dsp, (5′-GGCAGCTTGGCGGTCAGGAA-3′); D_{SP}^{int}, 270bp 5′ Dsp, (5′-CCGAATTCGTCCTCCAGAAACAGACC-3′); J_{H4}^{ext}, 190bp 3′ J_{H4} (5′-CCAGGGACTCCACCAACACC-3′); J_{H4}^{int}, 130bp 3′ J_{H4}, (5′-AAAGAATTCTAATCTGTCCTAAAGGCTC-3′). The amplification profiles for the primer sets were as follows: ext: 5′ @ 95C, (30″ @ 94C, 30″ @ 53C, 2.5′ @ 72C)$_{30\times}$, 5′; @ 72C. int: 5′ @ 95C, (30″ @ 94C, 30″ @ 50C, 2.5′ @ 72C)$_{20\times}$, 5′ @ 72C. The resulting amplified fragment was cloned and sequenced. The sequence was verified by using size-selected EcoRI-digested DNA containing the nonexpressed H chain gene rearrangement as a template for PCR amplification with the same set of nested primers.

Using the above approach, we were unable to recover the nonexpressed rearranged H chain alleles in hybridoma Spc4.3. Therefore, we resorted to genomic cloning of a size-selected fraction of EcoRI-digested DNA containing the nonexpressed H chain rearrangement. This was cloned in the manner previously described (18). The phage library and subsequent recombinant plasmids were screened for J_H-hybridizing sequence with a α[^{32}P]-labeled BamHI-EcoRI fragment of pJ11. A primer specific for a region 190 bp downstream of J_{H4} (5′-CCAGGGACTCCACCAACACC-3′) was used with Sequenase to obtain the sequence of the cloned fragment.

Database Search. All sequences were searched against the GCG Database using FastA (Genetics Computer Group [GCG] Sequence Analysis Package). L chain sequences were additionally searched against the database of Strohal et al. (26), kindly supplied by M. Weigert (Fox Chase Cancer Center). Sequence upstream of D_{Q52} was provided by H. Sakano (University of California, Berkeley, CA, unpublished results).

Assays for Antibody Autoreactivity. Hybridoma supernatants were

tested for reactivity to DNA, mouse thymocytes, and human epithelioid (Hep2) cells. The ELISA for anti-DNA reactivity was as described by Foster et al. (27). Supernatants were considered to react positively if the signal was >0.300 OD units above background (no supernatant). All supernatants were tested in duplicate at a 1:2 dilution. To screen for antithymocyte reactivity, dilutions of hybridoma supernatants were incubated with thymocytes from 5-wk-old BALB/c mice for 30 min on ice, washed three times with staining medium (deficient RPMI 1640, 3% FCS, 0.1% NaN_3), stained with fluorescein-coupled anti-IgM (331.12) for 20 min on ice, and washed again three times. After the final wash, thymocytes were resuspended in 200 μl of staining medium with propidium iodide and the cells were analyzed on a FACStar Plus®. Anti-nuclear/cellular reactivity was scored by immunofluorescence staining of Hep2 cells (Antibodies Inc., Davis, CA). Substrate slides contained wells with Hep2 cells, to which hybridoma supernatant was added. Slides were incubated for 30 min at room temperature in a moist chamber, washed with PBS, and stained with fluorescein-labeled goat anti-mouse IgM antibody (Fisher Scientific, Orangeburg, NY), at 10 μg/ml. The slides were washed, mounted with buffered glycerol, examined under a fluorescence microscope (Axiophot; Zeiss, Wetzlar, Germany), and photographed with TMax 400 film (Kodak).

Results

Peritoneal B Cells. Old BALB/c scid mice (≥1 yr of age), like old C.B17 scid mice (16), were found to contain detectable B cells ($B220^+IgM^+$) in their peritoneal cavity, though not in the spleen (splenic data not shown). The number of peritoneal B cells ranged from 2.7×10^4 to 1.2×10^6, representing between 1.7 to 34.5% of the harvested cells (in wild-type mice, B cell numbers ranged from 3 to 6×10^6). A striking feature of most scid B cells, illustrated in Fig. 1, is that they stained brightly and uniformly for IgM, whereas the staining of peritoneal B cells from control wild-type mice showed a wider range of intensity. One scid mouse (Fig. 1 *C*) appeared to contain populations of both B and pre-B ($B220^+IgM^-$) cells. None of the scid mice examined in Fig. 1 contained B cells with detectable expression of CD5 (data not shown), though $CD5^+$ B cells were noted previously in the peritoneal cavity of some old C.B17 scid mice (16).

DNA from peritoneal cells of individual old BALB/c scid mice showed a very restricted number of H chain gene rearrangements, in agreement with previous evidence that leaky scid mice contain few B cell clones (15, 28, and 29). As illustrated in Fig. 2 *A*, several mice showed only one prominent rearranged fragment, whose abundance in three of the mice (SpcB–D) was comparable to that of the germline fragment. This suggested, as confirmed below, that many IgM-producing clones might retain one H chain allele in germline configuration. Southern analysis of peritoneal cells from individual control mice also revealed a restricted number of H chain gene rearrangements (data not shown), in agreement with the previous results of Stall et al. (30). These investigators showed that peritoneal B cell populations are oligoclonal in old mice and that in many individuals such populations consist of predominantly $CD5^+$ or $CD5^-$ B cells.

Peritoneal B Cell Hybridomas. B cell clones from the peritoneal cavity of old mice were stimulated with *S. typhimurium* mitogen and fused with SP2/0 cells. 92 hybridomas were obtained in six separate fusions with scid cells and 38 hybridomas were obtained from four separate fusions with wild-type cells. IgM-secreting hybridomas were cloned and tested by Southern blot analysis for the status of their H chain genes.

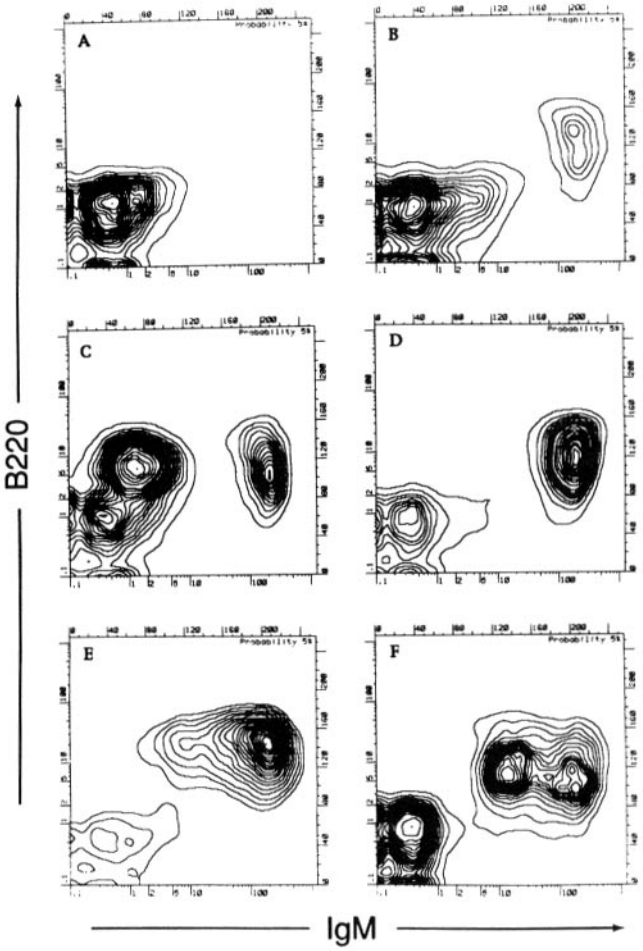

Figure 1. FACS® profiles of $B220^+IgM^+$ B cells from the peritoneal cavity of old individual BALB/c scid mice (*B–E*). Young adult BALB/c scid (3 mo of age) generally lack detectable peritoneal B cells, as illustrated in *A*. The FACS® profile of peritoneal B cells from an old BALB/c (+/+) mouse (*F*) is shown for comparison.

21 distinct scid B cell clones were identified based on the size of pJ11 (J_H) hybridizing fragments in EcoRI-digested DNA samples from 65 IgM-secreting hybridomas. The results are summarized in Table 1. Nine clones (examples are shown in Fig. 2 *B*) retained one H chain allele in germline configuration (R/G). This phenotype is highly unusual, as developing B cells generally show H chain gene rearrangements at both alleles (31). Five clones contained rearrangements at both H chain alleles: in two clones, the rearrangements (R/R) did not appear abnormal, but in three clones, one allele (R′) contained a grossly abnormal rearrangement that resulted in a loss of pJ11 hybridizing sequence. The latter three clones showed both a germline and nongermline $pC_\mu 3741$ hybridizing fragment. Thus, these clones apparently incurred an aberrant J_H-associated deletion on one H chain allele that ex-

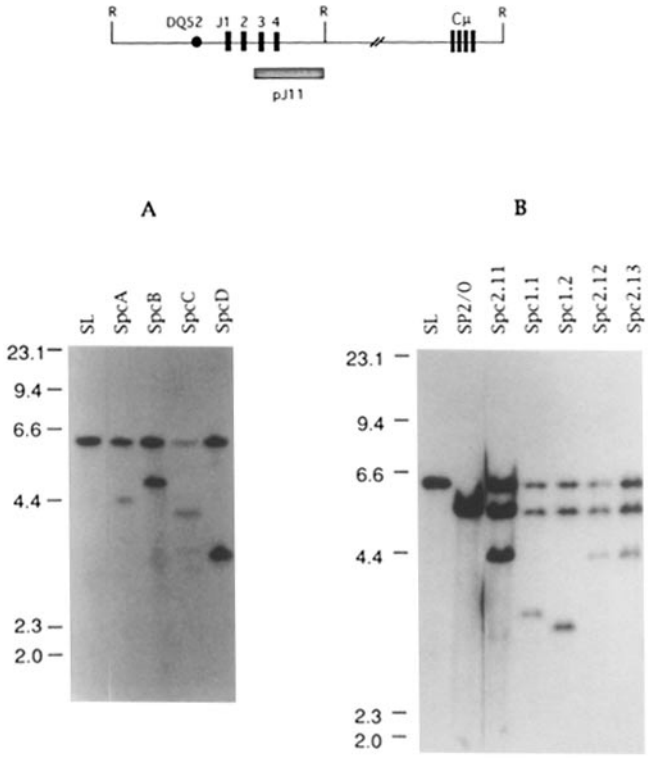

Figure 2. Southern blot analysis of H chain gene rearrangements in scid peritoneal B cells and peritoneal B cell hybridomas. Approximately 10 μg of DNA from each peritoneal cell sample was EcoRI digested, Southern blotted, and hybridized to a J_H-specific probe (α[^{32}P]dCTP-labeled pJ11). An EcoRI restriction map of the J_H-C_μ region is included for illustration. The position of the pJ11 hybridizing germline fragment is shown in lane *SL* (scid adult liver DNA). In *A*, the lanes *SpcA–D* contained DNA from peritoneal cells of individual mice (cells from mice corresponding to SpcB–D were used for fusion 1 in Table 1). *B* shows three examples of scid peritoneal B cell hybridomas with an R/G phenotype (*Spc2.11*, *2.12*, and *2.13* represent different isolates of the same clone). The contribution of the SP2/0 fusion partner is shown in lane SP2/0.

tended sufficiently downstream of J_{H4} to abolish the EcoRI site in the J_H-C_μ intron (see map in Fig. 2). Such deletions result in a nongermline pC_μ3741 hybridizing fragment and are often seen in transformed scid pre-B cells (9). Seven clones (R/−) contained only one pJ11 hybridizing allele and exhibited a single germline pC_μ3741 fragment. These clones may have lacked sufficient pJ11 hybridizing sequence due to an abnormal deletion that did not extend beyond the EcoRI site in the J_H-C_μ intron, or they may have simply lost one copy of chromosome 12. A comparable fraction of the wild-type hybridomas showed the same phenotype (see below), and we therefore arbitrarily designated these clones as R/−, with the (−) signifying a loss of chromosome 12.

14 of 31 IgM-secreting hybridomas from old wild-type mice were shown to be distinct (Table 1). Nine clones showed rearrangements at both H chain alleles (R/R) and one retained an allele in germline configuration (R/G). Four clones (R/−) showed only one J_H hybridizing fragment and a single germline pC_μ3741 fragment, and are presumed to have lost one copy of chromosome 12. Consistent with the known oligoclonality of peritoneal B cells in old BALB/c mice (30), very few distinct IgM-secreting clones (two to six) were recovered per cell fusion.

The status of the L_κ chain genes was more difficult to judge due to potential masking by the germline and rearranged L_κ chain loci of the SP2/0 hybridoma fusion partner

Table 1. *IgM-secreting Hybridomas from the Peritoneal Cavity of scid and Wild-Type Mice: Status of H Chain Alleles*

Mouse	Fusion	No. of IgM$^+$ hybridomas	No. of distinct clones and status of H chain alleles*			
			R/G	R/R	R/R′	R/−
scid	1‡	19	3	0	0	1
	2	10	1	0	0	1
	3	4	2	0	0	0
	4	4	0	1	1	1
	5	12	1	0	0	2
	6	16	2	1	2	2
Wild type	1	9	1	3	0	2
	2	5	0	3	0	1
	3	11	0	2	0	0
	4	6	0	1	0	1

* Status of H chain alleles was ascertained by Southern blot analysis with J_H- and C_μ-specific probes. R, rearranged (not grossly abnormal); R′, abnormally rearranged; G, germline, and −, probable loss of chromosome 12.
‡ Fusion 1 was with pooled peritoneal cells of three scid mice; all other fusions were with peritoneal cells of individual mice.

Hyb	V_H	N/P	D_H	N/P	J_H
Spc	**7183**				
1.2(1)	TAC TGT GC		1-C TAC TAT AGG TAC GAC	GTCGGG	8-GAC TAC(J_2)
3.2(1)	TAC TGT GCA AGA C	TT	2-TAC TAT GGT AAC TAC	GTCC	6-TT GAC TAC(J_2)
6.4(2)	TAC TGT GCA AGA		TCT AC-12		1-C TAC TTT GAC TAC(J_2)
6.5(u)	TAC TGT GCA AGA		TCT-14	G	3-GG TTT GCT TAC(J_3)
	Q52				
1.1(1)	TAC TGT GCC AAA	C	6-AT AGG TAC-3		2-TAC TAT GCT ATG GAC TAC(J_4)
4.1(2)	ACT GTG CCA AA	C	6-ATA GGT AC-3	AT	9-CT ATG GAC TAC(J_4)
4.3(1)	TAC TGT GCC AAA	C	6-AT AGG TAC-3		5-TAT GCT ATG GAC TAC(J_4)
	S107				
1.4(u)	TAC TGT GCA AGA GAT A	TATCTC		G	C TAC TGG TAC TTC CAT(J_1)
	Gam3.8				
5.3(3)	TTC TGT GCA AGA	T TAGTAGA	T CTA CTA TGG TT-5		7-T GCT TAC(J_3)
	J606				
3.3(u)	TAC TGT GCT AGA		5-TAT G-8		9-CT ATG GAC TAC(J_4)
6.6(2)	TAC TGT		TCT A-13	GG	5-TAT GCT ATG GAC TAC(J_4)
6.9(2)	TAC TGT GCT AGA		TCT A-13	G	7-T GCT ATG GAC TAC(J_4)
	V10				
6.3(2)	TAC TGT GTG AGA	CAT	15-GTA G-4	G	4-C TAT GCT ATG GAC TAC(J_4)
	J558				
1.3(u)	TTC TGT GCG AGA	TC		G	17-TAC(J_4)
2.1(u)	TAC TGC GCA AGA		TCT-14		2-TAC TAT GCT ATG GAC TAC(J_4)
5.1(u)	TTC TGT GCA AGA		TCT-14		8-GCT TAC(J_3)
5.5(1)	TAC TGT GCA A		TT TAT TAC TAC-12		5-TTT GAC TAC(J_2)
6.2(u)	TAC	CG			7-T GCT ATG GAC TAC(J_4)
6.7(2)	TAC TGT G		4-CT ATG G-7		15-AC TAC(J_4)
	VHU-1				
4.2(1)	TTC TGT GCA AGA	TC GA	TC TAC TAT GGT TAC-3		4-TGG TAC TTC GAT(J_1)

Figure 3. Sequence of VDJ coding junctions from scid peritoneal B cell hybridomas. Each hybridoma is designated with a two-digit number; the first digit refers to the cell fusion and the second designates the clonal isolate. The superscript numbers in parentheses indicate the D reading frame based on the nomenclature of Ichihara et al. (36); (*u*) the reading frame is unassigned. Sequences are arranged according to the known order of V_H genes, with the most J_H-proximal V_H genes at the top. Only V_H sequence encoding the CDR3 region is shown. Nucleotides common to D_H and V_H (or D_H and J_H) are arbitrarily assigned to D_H. The identity of the J_H element is shown within the parenthesis at the far right. The extent of D_H and J_H nucleotide loss is indicated by the numbers flanking these sequences. N and P additions are shown under N/P, with P nucleotides underlined. Nucleotides assigned to V_H, D_H, or J_H that could be potential P additions are also underlined. Hybridoma Spc2.2 (not shown for space considerations) contained a D_H-D_H fusion: V_H(J558), TATTACTGTGCA; N/P, GA; D_H, TCTAC; D_H, ATTG; N/P, CTAC, J_{H4}, GACTAC.

Hyb	V_H	N/P	D_H	N/P	J_H
Hpc	**7183**				
1.1(1)	TAC TGT GCA AGA C		3-AT TAC TAC GGT AGT AGC TAC		2-TAC TTT GAC TAC(J_2)
4.2(1)	TAC TGT GCA AGA C		3-AT TAC TAC GGT AGT AGC TAC		2-TAC TTT GAC TAC(J_2)
	Vh11				
1.3(1)	TTC TGT ATG AGA		5-TAT GGT AAC TAC		4-TGG TAC TTC CAT GTC(J_1)
2.1(u)	TAC TGT GCA AGA	GAGGAAGCT	1-AAC TGG GAC	GGGG	6-TT GAC TAC(J_2)
2.4(1)	TTC TGT ATG AGA		5-TAT GGT AAC T		4-TGG TAC TTC GAT GTC(J_1)
	36-60				
2.2(3)	TTC TGT GCA AGA	A	8-AG GTA CGA C	AT	TAC TAT GCT ATG GAC TAC(J_4)
2.3(1)	TAC TGT GCA AGA	AGGGCCC	3-AC TAT GAT TAC GAC	GGGACCGC	4-C TTT GAC TAC(J_2)
4.1(1)	TAC TGT GCA AGG	GGCTATG	3-AT TAC TAC-12	AGGA	3-AC TTT GAC TAC(J_2)
	Gam3.8				
1.2(2)	TAC TGT GCA AGA	TCGGCAGCTCG	11-G GCT AC	GTGGCGGGCAA	CC TGG TTT GCT TAC(J_3)
	J606				
1.7(1)	TAT TAC TGT GCT AG		4-G TAT G-8		9-CT ATG GAC TAC(J_4)
	J558				
1.6(u)	TAC TGT GCA AGA		TCT-14		2-TAC TAT GCT ATG GAC TAC(J_4)
3.1(1)	TAC TGT GCA AGA	TGGAG	7-T GGT AAC TAC	CCGT	AC TAC TTT GAC TAC(J_2)
3.2(1)	TAC TGT GCA AGA	GAGGG	3-C TAT GGT TAC-3	CCCC	6-TT GAC TAC(J_2)
	VHU-1				
1.5(1)	GAG GAT TCT GCA GTC		2-TAT TAC-15	CG	7-T GCT ATG GAC TAC(J_4)

Figure 4. Sequence of VDJ_H coding junctions from wild-type peritoneal B cell hybridomas. The format and symbols are as in Fig. 3.

(32). Thus, although many hybridomas contained only one novel nongermline fragment (data not shown), the status of the other L_κ chain allele was unclear; it may have been unrearranged, masked, or missing due to a loss of chromosome 6.

Structure of Expressed H and L_κ Chain Genes in IgM^+ Hybridomas. Rearranged H and L_κ chain genes in the IgM^+ hybridomas of Table 1 were PCR amplified from cDNA, cloned, and sequenced. Sequences of VDJ_H and VJ_κ recombination junctions are shown in Figs. 3–6. The extent of D and J nucleotide deletion in VD_H, DJ_H, and VJ_κ junctions is indicated by the numbers flanking these sequences. Nucleotides that could be encoded by either D_H or J_H and V_H or D_H have been arbitrarily assigned to the D_H region. Nontemplated (N)[1] base additions (5), and templated (P) base additions that are palindromic to unmodified coding ends (33,

[1] *Abbreviations used in this paper:* N, nontemplated; P, templated.

Hyb	Vκ	N/P	Jκ
Spc	Vκ21		
3.3	CAG CAA AGT AAG GA	CA	TG TAC ACG TTC GGA(J2)
	Vκ8		
5.3	CAG CAA TAT TAT		5-ACG TTC GGA(J2)
6.2	CAT CAA TAC CTC TCC T	C	G TGG ACG TTC GGT(J1)
6.3	CAT CAA TAC CTC TCC T	C	G TGG ACG TTC GGT(J1)
6.7	CAT CAA TAC CTC	GTC CT	G TGG ACG TTC GGT(J1)
	Vκ19		
2.1	CAG CAA CAT TAT AGC ACT CC		A TTC ACG TTC GGC(J4)
	Vκ4/5		
1.1	CAG CAG TGG AGT AGT AAC CCA CCC A	C	G TGG ACG TTC GGT(J1)
1.2	CAG CAA AGG AGT AGT TAC CCA CCC A	C	G TGG ACG TTC GGT(J1)
1.3	CAG CAG TTT ACT ACT TCC	AC	G TGG ACG TTC GGT(J1)
1.4	CAA CAG TGG AGT AGT TAC CCA	CTG	5-ACG TTC GGA(J2)
3.2	CAG CAG TGG AGT AGT AAC CCA CCC A	C	G TGG ACG TTC GGT(J1)
4.1	CAG CAG TAC AGT GGT TAC CCA TCC A	C	G TGG ACG TTC GGT(J1)
4.2	CAA CAG TGG AGT AGT TAC CCA	CT	4-C ACG TTC GGA(J2)
* 4.3	CAA CAG TGG AGT AGT AAC CCA C		G TGG ACG TTC GGT(J1)
5.5	CAG CAG TGG XGT AGT AAC CCA CCC A	C	G TGG ACG TTC GGT(J1)
* 6.3	CAG CAA TGG AGT AGT AAC CAC	CAC	G TGG ACG TTC GGT(J1)
6.4	CAG CAG TTT ACT AGT TCC	AC	G TGG ACG TTC GGT(J1)
6.6	CAG CAG TTT ACT AGT TCA CT		G TGG ACG TTC GGT(J1)
6.9	CAG CAG TGG AGT AGT AAC CCA CCC A	C	G TGG ACG TTC GGT(J1)
	Vκ1		
2.2	TTA CAA GGT ACA CAT CAG CC		1-G TAC ACG TTC GGA(J2)
	Vκ9		
4.3	CTA CAG TAT GAT AAT CTT CTA CC	GGTAGAA TCCAC	G TGG ACG TTC GGT(J1)
5.1	CTA CAG CAT GGT GXG AGC	CC	G TGG ACG TTC GGT(J1)
6.5	GTA CAG TAT GCT GAG T	CCAC	G TGG ACG TTC GGT(J1)

Figure 5. Sequence of VJ_κ coding junctions from scid peritoneal B cell hybridomas. The format is as in Fig. 3. Sequences are arranged according to the known order of V_κ genes, with the most J_κ-proximal V_κ genes at the top. Nucleotides that could be either V_κ coding sequence or J_κ-derived P additions have been arbitrarily assigned to V_κ. Also included in this figure are the rearranged but nonexpressed alleles of hybridomas Spc4.31 and Spc6.31 (marked with an asterisk).

34), are denoted under the heading N/P (P nucleotides are underlined). Additional details are given in the figure legends.

H Chain Genes. A comparison of Figs. 3 and 4 reveals a number of interesting differences between the expressed H chain genes of scid and wild-type hybridomas. (*a*) In the scid hybridomas there is over-representation of J_{H4} (12/21 scid vs. 4/14 wild-type hybridomas). A similar over-representation of J_H has been seen in neonatal B cells of BALB/c mice (35). (*b*) In six scid hybridomas (Spc3.3, 4.2, 4.3, 5.3, 5.5, and 6.7), the D_H-J_H junctional sequences contain three to five nucleotides that may have been encoded by either the D_H or J_H element (illustrated in Fig. 7). Thus, here the recombination appears to have occurred between short homologies; e.g., TGGTT in clone Spc5.3 and ACTAC in clone Spc5.5. No homology-based D_H to J_H joining is evident in the wild-type hybridomas. (*c*) The extent of D_H nucleotide deletion is greater in scid than wild-type hybridomas. 7 of 21 scid hybridomas (vs. 1 of 14 wild-type hybridomas) retained only three to four nucleotides assignable to D_H. In three hybridomas (Spc1.3, 1.4, and 6.2), the D_H element was apparently deleted or not used. The latter possibility is discussed later and may apply to three additional scid hybridomas (Spc2.1, 5.1, and 6.9), where the "D_H nucleotides" (TCT or TCTA) are palindromic to the coding ends of the adjacent V_H gene. These nucleotides could represent V_H-derived P additions. Note that scid hybridomas Spc1.4 and 5.3 show unusually long P additions. (*d*) In the 13 scid VDJ_H junctions with four or more nucleotides assignable to a D_H element (excluding Spc6.9), five show the D_H in reading frame 2 (using the nomenclature of Ichihara et al. [36]). (*e*) The VD_H and DJ_H junctions of the scid hybridomas entirely lack N additions or contain N additions of only one to two nucleotides (with one exception, Spc6.3). The wild-type hybridomas fall

Hyb	Vκ	N/P	Jκ
Wpc	Vκ19		
1.6	CAG CAA CAT TAT AGC ACT CC		A TTC ACG TTC GGT(J4)
	Vκ10		
4.2	CAA CAG GGT AAT ACG CT		G TGG ACG TTC GGT(J1)
	Vκ4/5		
1.1	CAA CAG CAT AAT GAA TAC CCG	TAT	G TGG ACG TTC GGT(J1)
1.2	CAG CAG TGG AGT AGT AAC CAC	ACCAC	G TGG ACG TTC GGT(J1)
2.2	CAG CAG TGG AGT AGT AAC CC		G CTC ACG TTC GGT(J5)
3.2	CAT CAG TGG AGT AGT TAT C		3-AC ACG TTC GGA(J2)
	Vκ1		
1.5	TCT CAA AGT ACA CAT GTT CC		G TGG ACG TTC GGT(J1)
2.3	TTA CAA GGT ACA CAT CAG CC		G TGG ACG TTC GGT(J1)
4.1	TTA CAA GGT ACA CAT CAG CC		1-G TAC ACG TTC GGT(J2)
	Vκ9		
1.3	CTA CAG CAT GGT GAG AGC CC		A TTC ACG TTC GGC(J4)
2.1	CTA CAG TAT GAT AAT CTT C		2-GG ACG TTC GGT(J1)
2.4	CTA CAG CAT GGT GAG AGC CC		A TTC ACG TTC GGC(J4)

Figure 6. Sequence of VJ_κ coding junctions from wild-type peritoneal B cell hybridomas. The format and symbols are as in Fig. 5.

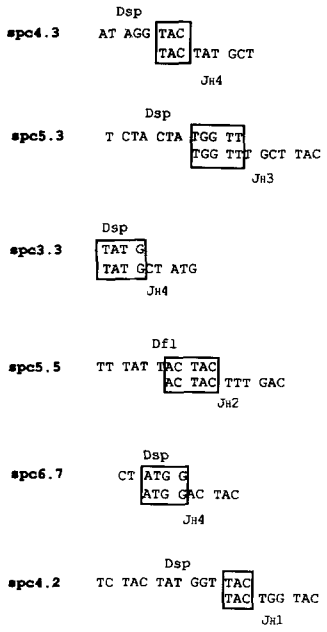

Figure 7. DJ_H recombination between short regions of homology in scid peritoneal B cell hybridomas. Junctional sequences that may have been encoded by either the D_H or J_H element are shown within the boxes and are taken from Fig. 3.

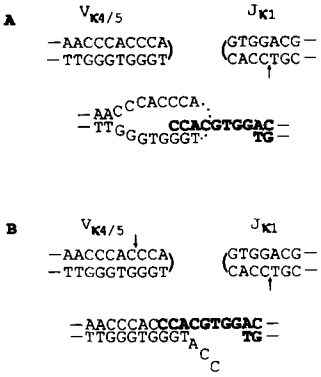

Figure 8. Two possible models for homology-directed joining of $V_{K4/5}$ and J_{K1} coding ends. Details are given in the text.

into two classes: those with N additions (four to nine nucleotides) at both VD_H and DJ_H junctions, and those lacking N additions at both junctions (with two exceptions, Npc1.5 and 2.2).

L Chain Genes. The VJ_K junctions of the expressed L_K chain genes in scid and wild-type hybridomas are shown in Figs. 5 and 6 (the junctions of two nonexpressed L_K chain gene rearrangements, marked with an asterisk, are also shown in Fig. 5). The striking feature of the scid results is the high frequency of N/P additions. N additions are present in 3 junctions (Spc1.4, 4.2, and 6.7) and P additions are seen in 15 junctions. One junction (from Spc4.3) contains unusually long P additions derived from both coding elements. In contrast to the scid hybridomas, only 2 of 14 wild-type hybridomas show N/P additions (Fig. 6).

Of particular interest are the eight scid $V_{K4/5}$-J_{K1} junctions that appear to represent recombinations between short stretches of homology shared between J_{K1}-derived P additions and $V_{K4/5}$ members (the assignment of the underlined $V_{K4/5}$ nucleotides is arbitrary as these could correspond to germline sequences of the $V_{K4/5}$ gene family or represent P nucleotides derived from J_{K1}). Two possible models for homology-directed joining of $V_{K4/5}$ and J_{K1} coding ends are illustrated in Fig. 8. The first (Fig. 8 *A*) involves nicking at a variable distance from one hairpin terminus (J_{K1}) and entrance of the protruding strand between the separated strands of the other terminus ($V_{K4/5}$). Pairing of the P nucleotides (e.g., CCA) from J_{K1} with the complementary terminal nucleotides (GGT) of $V_{K4/5}$ might help resolve the hairpin at $V_{K4/5}$. In the second model (Fig. 8 *B*), both coding ends are nicked at a variable distance from their hairpin termini, and the protruding P nucleotides from one strand (J_{K1}) are paired with complementary nucleotides on the opposite strand of the other element ($V_{K4/5}$), producing a "flap-gap'" intermediate (37). Ligation of abutted ends and repair synthesis would follow the pairing event in both models.

Structure of Nonexpressed Rearranged H Chain Genes in Two scid Hybridomas with a H Chain Gene Status of R/R (Table 1). Two scid hybridomas (Spc4.3 and 6.3) appeared to represent potential revertants since their nonexpressed rearranged H chain alleles did not appear grossly abnormal by Southern analysis. However, the cloning and sequencing of the nonexpressed H chain alleles in these hybridomas revealed scid-like abnormalities (Fig. 9). In hybridoma Spc4.3, an apparent attempted joining of D_{Q52} to J_{H4} resulted in deletion of D_{Q52} and ~1 kb of the upstream sequence. In hybridoma Spc6.3, J_{H2} was joined to a site 72 bp upstream of $D_{SP2.2}$. Another abnormal recombination also apparently occurred and resulted in a breakpoint 9 bp downstream of J_{H2} and 107 bp downstream of J_{H4}. The possibility remains, however, that a reversion event could have occurred during the period of L_K chain gene rearrangement, because the nonexpressed L_K chain alleles in Spc4.3 and Spc6.3 were rearranged normally (see Fig. 6).

Screen of Peritoneal B Cell Hybridomas for Autoreactivity. Previous studies have shown that the B cell repertoire of aging mice, including BALB/c mice, becomes increasingly skewed toward reactivity against self-antigens (38). Therefore, we screened culture supernatants from scid and wild-type hybridomas for potential anti-nuclear/cellular reactivity using human epitheloid (Hep2) cells (39). 17 of 20 scid hybridoma supernatants examined reacted with Hep2 cells; 5 of these supernatants also showed reactivity to single-stranded DNA

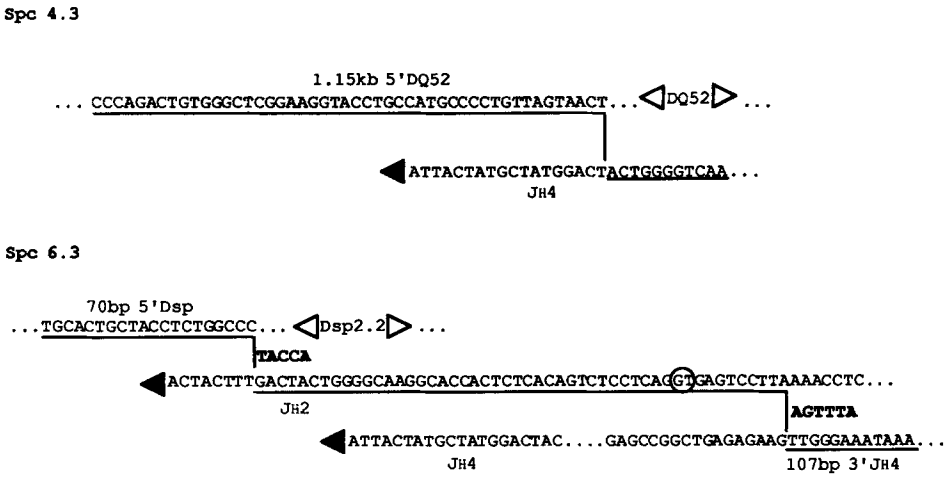

Figure 9. Sequence of recombination junctions in the nonexpressed H alleles of scid hybridomas Spc4.3 and 6.3. Recombined sequences are underlined, with the breakpoints indicated by vertical lines and N additions in bold to the right of the vertical lines. Signal sequences with 12- and 23-bp spacers are denoted by open and filled triangles, respectively. The J_{H2} splice site (GT) in Spc6.3 is circled.

(ssDNA), double-stranded DNA (dsDNA), or to BALB/c thymocytes. 7 of the 14 wild-type hybridoma supernatants reacted with Hep2 cells; the positive supernatants (with one exception) also showed reactivity to one or more of the other antigens (ssDNA, dsDNA, and BALB/c thymocytes).

Different immunofluorescence staining patterns were observed with Hep2 cells. One pattern showed prominent staining of the cytoskeleton (Fig. 10 *A*) and was observed with three scid hybridomas (Spc1.2, 3.2, and 6.4) and with two wild-type hybridomas (Npc1.1 and 4.2). All five of these hybridomas express 7183 V_H genes and J_{H2}. Another pattern consisted of cytoplasmic and nuclear staining with nucleoli unstained; cells in mitosis were brightly stained (Fig. 10 *B*). This pattern was seen with three hybridomas that express J558 V_H genes and J_{H4} (Spc1.3 and 2.3, and Npc1.6) and with a fourth hybridoma that expresses a V_H10 gene and J_{H4} (Spc6.3). No restricted V_κ chain gene expression was associated with either of the above staining patterns. A third pattern, the smooth dense nuclear staining in Fig. 10 *C*, was seen with hybridoma Spc1.4.

Discussion

B Cell Leakiness in scid Mice Appears to Result from a Low Frequency of V(D)J Coding Joint Formation. Previous studies have indicated that V(D)J coding joint formation can occur

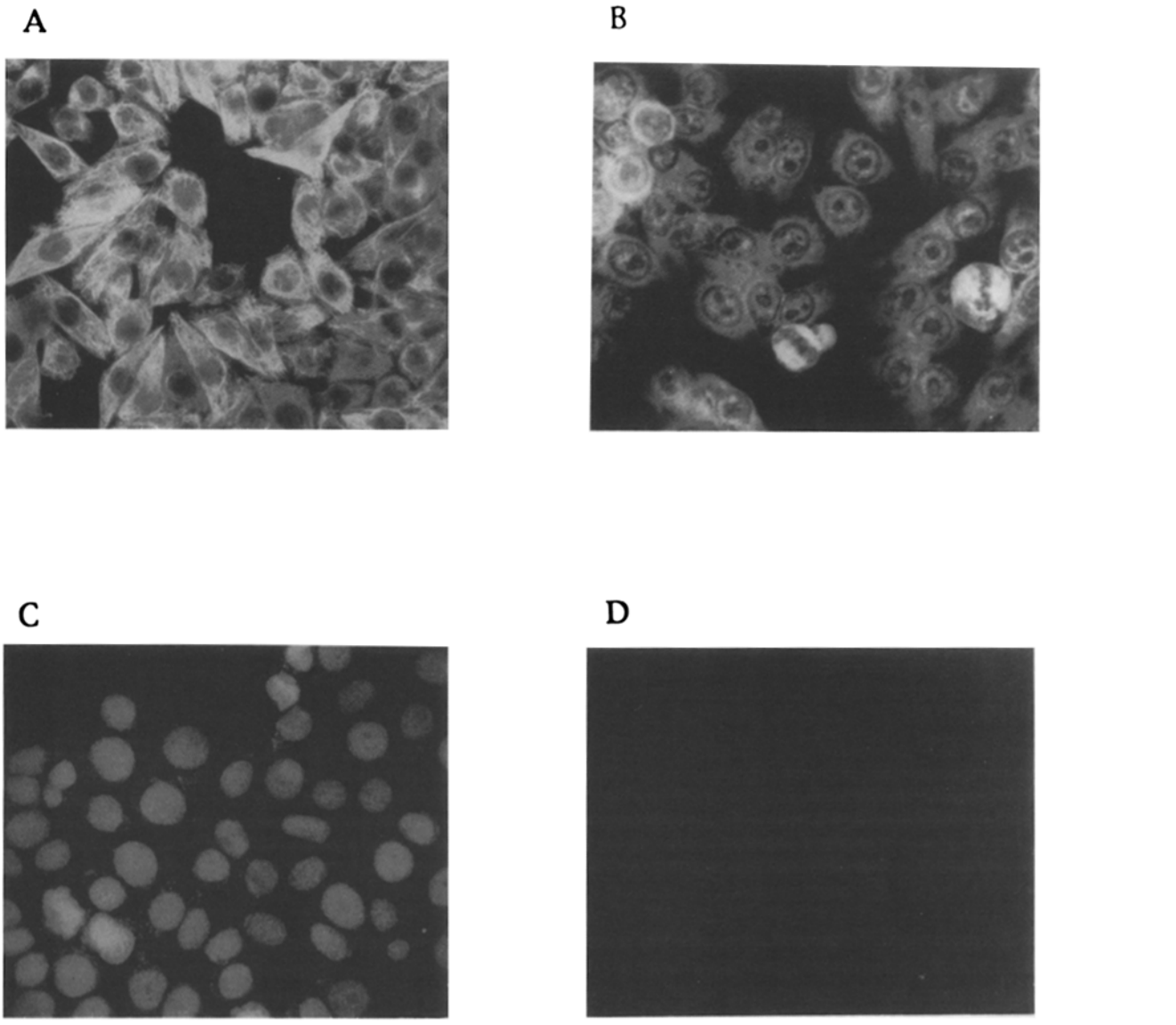

Figure 10. Common immunofluorescence staining patterns of Hep2 cells by supernatants of independent scid and wild-type B cell hybridomas. (*A*) Cytoskeletal staining by Spc3.2 (also seen with Spc6.4, 1.2, Npc1.1, and 4.4). (*B*) Nuclear, cytoplasmic, and mitotic figure staining with nucleoli unstained by Spc6.3 (also seen with Spc2.3, 1.3, and Npc1.6). (*C*) Smooth dense nuclear staining by Spc1.4. (*D*) Unreactive control supernatant (Npc4.1).

at low frequency in recombinase-defective B lineage cells of scid mice (12, 18, and 19). To test whether this might account for scid B cell leakiness, we analyzed H and L_κ chain genes in peritoneal B cell hybridomas recovered from old leaky scid mice. We reasoned that survival of developing scid B lymphocytes might depend on their making VDJ_H and VJ_κ coding joints with a minimal number of attempts, presuming that most such attempts result in cell death. Accordingly, the nonexpressed H chain allele in leaky scid cells would be expected to show either no rearrangement or an abnormal rearrangement, similar to that seen in transformed scid pre-B cells (9). Consistent with the above, 9 of 14 informative hybridomas retained the nonexpressed H chain allele in germline configuration. The remaining five hybridomas contained abnormal rearrangements at their nonexpressed H chain allele: in three hybridomas (Spc4.1, 6.2, and 6.4), the rearrangements were grossly abnormal as ascertained by Southern analysis, and in two hybridomas (Spc4.3 and 6.3), the abnormal rearrangements were discernible by sequence analysis (Fig. 9).

In contrast to the above, wild-type B cells generally show normal rearrangements at both H chain alleles (25, 40). H chain gene rearrangement is initiated during the pro-B and early pre-B cell stages of differentiation (31, 41, 42); L_κ chain rearrangement is observed in the late pre-B cell stage (41, 42). This developmental sequence could reflect an intrinsically higher frequency of V(D)J recombination at the H than the L_κ chain locus (25), or an ordered control of H and L_κ chain gene rearrangement (reviewed in reference 43). If an ordered regulatory mechanism is operative, the control cannot be absolute, because as shown in mouse and other species, L_κ chain gene rearrangement can occur independently of H chain gene rearrangement (44–46). Recently, L_κ chain gene rearrangement has been detected in pro-B cells ($B220^+$ $CD43^+$) of mice lacking a functional H chain locus (47). This has been interpreted (47) to indicate that L_κ chain gene rearrangement occurs at low frequency in pro-B cells, independently of a much higher frequency of H chain gene rearrangement. Accordingly, some (rare) pro-B cells could be expected to make a productive VDJ_H and VJ_κ rearrangement without having yet made a rearrangement at their other H and L_κ chain alleles. In scid mice, there would be a strong selection for such cells, presuming that most attempted Ig gene rearrangements result in cell death. We suggest that this could account for the high frequency of scid B cell hybridomas with the nonexpressed H chain allele in germline configuration.

Further evidence of the defective scid recombinase activity was seen in the expressed H (and L_κ) chain alleles of the scid B cell hybridomas. First, many H chain junctions contained no more than four assignable D_H nucleotides or entirely lacked D_H nucleotides. This could reflect excessive deletion by the scid recombinase activity (9). Alternatively, in those rearranged alleles lacking D_H nucleotides, it is possible that a D_H element was not even involved in the rearrangement. There were no D_H nucleotides in the H chain junctions of hybridomas Spc1.3, 1.4, and 6.2, and in hybridomas Spc2.1, 5.1, and 6.9, the assigned D_H nucleotides probably represent V_H-derived P additions. Possibly, a productive V_H to J_H joining occurred in these hybridomas in violation of the 12/23 rule (reviewed in reference 1). The rule is that joining of any two coding elements to one another requires that they be flanked by recombination signal sequences with different length spacers (12/23 rule); V_H and J_H elements are each flanked by recombination signal sequences with 23-bp spacers. However, examples of H chain rearrangement that violate the 12/23 rule have been reported by several laboratories (48–50). If this applies in the present case, one could again argue that survival of developing scid B cells depends on their making productive V(D)J rearrangements with a minimal number of attempts, and that there is a very strong selection for rare B cells with productive V_H to J_H rearrangements. Another way to account for the absence of a D_H element in the above junctions is to postulate the formation of a hybrid joint (6, 10) between the signal sequence 5′ of a given D_H element and the respective J_H elements; subsequent joining of the J_H elements with a V_H element would not violate the 12/23 rule. Second, unusually long P additions of four to seven nucleotides were observed in many VDJ_H and VJ_κ scid junctions (Figs. 3 and 6). A high frequency of long P additions has been previously noted in coding joints recovered from rearranged TCR genes in nontransformed scid thymocytes (51) and in scid thymic lymphomas (20). Extensive characterization of scid thymic lymphomas has shown that they express (or show clear evidence of having expressed) the defective scid recombinase activity (9, 10, 20).

None of the scid B cell hybridomas examined showed evidence of a reverted H chain gene phenotype; e.g., none contained a nonexpressed H chain allele that was normally rearranged. However, in the two hybridomas for which we obtained junctional sequences of the nonexpressed L_κ alleles, both alleles were rearranged normally. This result is not expected for cells that express the defective scid recombinase activity as reasoned in the beginning of this paper and elsewhere (17). Thus, this leaves open the possibility that a reversion event could have occurred during the period of L_κ chain gene rearrangement in the cells represented by these two hybridomas.

An apparent example of reversion in a recombinase-active scid pre-B cell was reported by us recently (18). We recovered a transformed pre-B cell clone from the peritoneal cavity of a leaky scid mouse that expresses wild-type recombinase activity and contains two normal VDJ_H rearrangements: one allele (VDJ_H^+) encodes a μ chain, the other is nonproductively rearranged (VDJ_H^-). Additional evidence for reversion of the scid phenotype has been reported for scid T cells (17, 52). Three of five cloned T cell lines from individual leaky scid mice were found to contain normal rearrangements at both expressed and nonexpressed T cell receptor loci. 9 of 12 analyzed rearrangements in these three cell lines (1280, 1287, and 7167) correspond to nonexpressed loci. All nine appear normal by Southern analysis, and in seven cases, this has been confirmed by sequencing the recombination junctions. With several examples of T cell lines showing a reverted scid phenotype, we cannot easily explain the apparent absence of revertants in the present sample of scid B cell hy-

bridomas. One might argue that, given the differences in organization and potential number of recombinations of TCR and Ig genes (reviewed in reference 53), more attempted V(D)J rearrangements (deleterious events) would be expected to occur in developing T cells than B cells. This could result in the development of fewer scid T cells than B cells and in a very strong selection for T lineage revertants, as these would be the cells most likely to survive. An absence of the appropriate T cells could limit the manifestation of B cell leakiness. Indeed, T cell–dependent "rescue" of latent B cells in young adult scid mice has been reported (54, 55).

Recombination of V(D)J Coding Ends in Peritoneal scid B Cells Often Occurs between Short Stretches of Homology. In about half of the scid V(D)J coding junctions that lacked N addition, there were three or more nucleotides that could have been encoded by either of the participating coding elements (DJ_H, VD_H, or VJ_κ). This indicates that these recombinations occurred between short stretches of sequence homology. In six hybridomas, the recombination of D_H to J_H occurred between a three to five–nucleotide repeat (see Fig. 5). In all other cases of recombination involving short homologous sequences, one of the sequences resulted from a P addition. In hybridomas Spc6.9, 1.3, 2.1, and 5.1, the joining of V_H to D_H occurred within a three to four–nucleotide repeat, (T)AGA or TCT(A), depending on whether one assigns the P-based homology to the D_H or V_H element (see Fig. 3). The most striking example of recombination involving P-based homologies was seen in 8 of 11 productive rearrangements of $V_{\kappa 4/5}$ to $J_{\kappa 1}$. This was also seen in the two nonproductive $V_{\kappa 4/5}$ to $J_{\kappa 1}$ rearrangements of scid hybridomas Spc4.3 and 6.3 (see Fig. 5). Other examples of V(D)J recombination involving P-based homologies of three to five nucleotides have been recently reported for rearranged TCR γ and δ genes in T cells from fetal and newborn mice (56).

Junctional nucleotides that could derive from either coding element were first noted in DJ_H coding junctions lacking N nucleotides (36, 57). Recent analyses of DJ_H coding junctions from pre-B cells of neonatal mice, where N nucleotides are generally absent, have shown that most such junctions result from recombination between short regions of homology (58, 59). These findings have been interpreted to indicate that short homologous sequences facilitate the alignment and joining of coding ends (58, 59). This possibility was suggested earlier (37) based on studies showing that illegitimate recombination of DNA ends with 3′ or 5′ extensions involves short nucleotide homologies (60 and references therein). An alternative interpretation is that V(D)J junctions resulting from recombination between short regions of homology are developmentally selected based on the amino acid sequence that they encode. This idea has been suggested (34, 61) as a possible explanation for the restricted junctional diversity in rearranged TCR δ and γ genes of fetal and neonatal thymocytes (62, 63), where most junctions result from recombination between short regions of homology (34, 56, 61). A similar idea was put forward to explain the predominance of a particular $V_{L1}J_L$ junction recovered from B lineage cells in adult Muscovy ducks (64). This junction resulted from recombination between a 9-bp region of interrupted homology and was found to increase dramatically from days 21 through 35 of development.

While the possible role of short sequence homologies in V(D)J recombination has been controversial, recent studies show that such homologies may bias recombination and limit coding joint diversity. To test for the potential importance of developmental (thymic) selection, a construct with an unrearranged J_γ-$C\gamma$ gene segment and three unrearranged V_γ genes, each with a termination codon in their coding regions, was used to generate transgenic mice (65). VJ_γ junctions resulting from rearrangement of the transgene were then analyzed. In a second test system (56), the C_δ segment was disrupted at both TCR δ alleles, and the V(D)J junctions in the rearranged TCR δ and γ genes of these mice were examined. Although developmental or cellular selection for a particular γ protein or $\gamma\delta$ heterodimer was abrogated in both test systems, the same restricted V(D)J junctional diversity resulting from recombination between homologous sequences was observed as in productively rearranged TCR γ and δ genes of fetal and neonatal thymocytes. These results support the notion that short homologous sequences at the ends of coding elements may facilitate their alignment and joining. Further support comes from an analysis of coding joints generated on extrachromosomal V(D)J substrates in transfected cell lines (66). In this system as well, coding joint diversity was found to be severely restricted when coding ends contained short regions of homology. The maximum usage of homology in coding joint formation occurred in cell lines with low terminal deoxynucleotidyl transferase activity.

Given the findings discussed above and the low frequency of N addition in scid VDJ_H coding junctions, it is perhaps not surprising that many scid recombinations occurred between short stretches of homologous sequence. But, how does one account for the high frequency of recombinations involving P-based homologies? As discussed elsewhere (14), *scid* may impair the ability of the V(D)J recombinase activity to resolve hairpin structures at the termini of cleaved coding elements. Resolution of these structures may normally involve single strand nicks a few base pairs from the hairpin terminus (14, 53). This would explain the frequent occurrence of short P additions (1–2 bp) at unmodified coding ends (33, 34) in V(D)J junctions of wild-type cells. In the case of scid cells, hairpin termini may be either generally inaccessible to nicking or the nicking enzyme(s) could be defective or missing. Consequently, if single strand nicks occur at all, they may occur randomly at variable distances from the hairpin termini. This could account for the long P additions in scid V(D)J junctions (20, 51, and this study). The generation of overhangs at scid coding ends, together with the relative absence of N addition in scid B cells (discussed below), would presumably increase the probability of alignment between short regions of homology, and this could explain the high frequency of recombination involving P-based homologies.

Possible Origin and Specificity of scid B Cells. Previous studies have shown that the only lymphoid site where scid B cells can be routinely detected is the peritoneal cavity of old mice

(16). The spleen and lymph nodes of leaky scid mice generally lack detectable B cells (16, and unpublished results of G. Bosma). Another notable feature of scid B cell populations is that they are oligoclonal and long lived (15, 28, 29). In most individual leaky scid mice, only a few B cell clones account for all the detectable serum Ig, and often the same clones persist for many months in the absence of detectable recruitment of new clones (15, 28). The plasma cell progeny of these clones, which appear as irregularly scattered plasmacytic foci in the peripheral lymphoid tissues (15), could represent expanded progeny of oligoclonal peritoneal B cells. The oligoclonal nature of scid peritoneal B cells is clear from the results of this study. Given the long-lived potential of scid B cell clones, the apparent restricted location of scid B cells to the peritoneal cavity, and the high level of IgM expressed by these cells, one is led to ask whether scid B cells belong to the $CD5^+$ population of B cells. This population represents a self-renewing class or lineage of B cells that arises early in ontogeny, expresses high levels of IgM and low levels of IgD, and resides predominantly in the peritoneal cavity of adult mice (reviewed in references 67 and 68). Also present in the peritoneal cavity is a $CD5^-$ population of B cells with properties similar to $CD5^+$ peritoneal B cells (69). Both of these populations may belong to the same class or lineage of B cells and are referred to as B-1 cells (70).

Evidence consistent with the idea that scid peritoneal B cells might represent B-1 cells comes from our finding of low N addition in VDJ_H coding junctions of scid peritoneal B cell hybridomas. A low frequency of N addition is characteristic of fetal and neonatal B cells (58, 59). As B-1 cells appear early in ontogeny (71, 72) and have the capacity for self-renewal (73), most adult B-1 cells may be direct descendants of fetal precursors. In support of this idea, pro-B cells from wild-type fetal liver, but not from adult bone marrow, readily reconstitute scid mice with B-1 cells (74). If a large proportion of the adult B-1 cell population derives directly from fetal and neonatal B cell precursors, one would expect the VDJ_H junctions of many B-1 cells to show low N addition. This clearly is the case for B-1 cell lymphomas of adult mice. 6 of 11 such lymphomas were found to contain no N addition in their DJ_H and VD_H junctions, and 3 showed N addition only at their VD_H junction (75). Similar results were obtained in this study with the scid peritoneal B cell hybridomas and approximately half of the wild-type control hybridomas. An alternative interpretation of the low N addition in scid VDJ junctions is that it simply reflects a strong selection for cells with low terminal deoxynucleotidyl transferase activity regardless of their origin.

Another property common to B-1 cells is that the IgM secreted by these cells often shows autoreactivity (reviewed in reference 76). Evidence for autoreactive scid B cell clones was first reported by Kearney et al. (29) in a study of B cell hybridomas from the spleen of leaky scid mice. The present analysis of scid B cell hybridomas from the peritoneal cavity also indicates potential autoreactivity among scid B cell clones. First, 17 of 20 scid hybridoma supernatants were found to react with cellular and/or nuclear antigens in the Hep2 cells. A number of scid and wild-type hybridoma supernatants gave one of two distinct staining patterns with Hep2 cells. One staining pattern was associated with hybridomas that expressed a 7183 V_H gene and J_{H2}; the other was associated with hybridomas that expressed a J558 V_H gene and J_{H4}. Interestingly, the 7183 V_H gene family has been previously shown to be over-represented among poly/autoreactive antibodies of 6-d-old BALB/c mice (77). Second, in 5 of the 13 scid hybridomas (excluding hybridoma Spc6.9) that retained 4 or more D_H nucleotides, the D_H element was in reading frame 2. In V(D)J junctions of antibodies from normal mice, D_H usage of reading frame 2 is underrepresented (<5%) (78, 79). However, in a panel of poly/autoreactive antibodies from autoimmune mice, the D_H usage of reading frame 2 was found to be comparable to the D_H usage of reading frames 1 and 3 (80). We conclude that many scid B cell clones may be autoreactive.

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References

1. Tonegawa, S. 1983. Somatic generation of antibody diversity. *Nature (Lond.).* 302:575.

2. Lewis, S., A. Gifford, and D. Baltimore. 1985. DNA elements are asymmetrically joined during the site-specific recombination

of kappa immunoglobulin genes. *Science (Wash. DC).* 228:677.
3. Akira, S., K. Okazaki, and H. Sakano. 1987. Two pairs of recombination signals are sufficient to cause immunoglobulin V-(D)-J joining. *Science (Wash. DC).* 238:1134.
4. Hesse, J.E., M.R. Lieber, M. Gellert, and K. Mizuuchi. 1987. Extrachromosomal DNA substrates in pre-B cells undergo inversion or deletion at immunoglobulin V-(D)-J joining signals. *Cell.* 49:775.
5. 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.
6. Lewis, S.M., J.E. Hesse, K. Mizuuchi, and M. Gellert. 1988. Novel strand exchanges in V(D)J recombination. *Cell.* 55:1099.
7. Morzycka-Wroblewska, E., F. Lee, and S. Desiderio. 1988. Unusual immunoglobulin gene rearrangement leads to replacement of recombination signal sequences. *Science (Wash. DC).* 242:261.
8. Roth, D.B., P.B. Nakajima, J.P. Menetski, M.J. Bosma, and M. Gellert. 1992. V(D)J recombination in mouse thymocytes: double-strand breaks near T cell receptor δ rearrangement signals. *Cell.* 69:41.
9. 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.
10. 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.
11. 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 (Eur. Mol. Biol. Organ.) J.* 8:735.
12. Hendrickson E.A., M.S. Schlissel, and D.T. Weaver. 1990. Wild-type V(D)J recombination in *scid* pre-B cells. *Mol. Cell. Biol.* 10:5397.
13. Harrington, J., C.-L. Hsieh, J. Gerton, G. Bosma, and M.R. Lieber. 1992. Analysis of the defect in DNA end joining in the murine *scid* mutation. *Mol. Cell. Biol.* 12:4758.
14. Roth, D.B., J.P. Menetski, P.B. Nakajima, M.J. Bosma, and M. Gellert. 1992. V(D)J recombination: broken DNA molecules with covalently sealed (hairpin) coding ends in scid mouse thymocytes. *Cell.* 70:983.
15. 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.
16. Carroll, A.M., R.R. Hardy, and M.J. Bosma. 1989. Occurrence of mature B (IgM^+,$B220^+$) and T ($CD3^+$) lymphocytes in scid mice. *J. Immunol.* 143:1087.
17. Petrini, H.J-P., A.M. Carroll, and M.J. Bosma. 1991. 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.
18. Kotloff, D.B., M.J. Bosma, and N.R. Ruetsch. 1993. Scid mouse pre-B cells with intracellular μ chains: Analysis of recombinase activity and IgH gene rearrangements. *Int. Immunol.* 5:383.
19. 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 Vh to DJh rearrangements in pre-B cell lines from scid mice. *J. Exp. Med.* 171:1909.
20. Schuler, W., N.R. Ruetsch, M. Amsler, and M.J. Bosma. 1991. Coding joint formation of endogenous T cell receptor genes in lymphoid cells from scid mice: Unusual P-nucleotide additions in VJ-coding joints. *Eur. J. Immunol.* 21:589.
21. Bosma, G.C., R.P. Custer, and M.J. Bosma. 1983. A severe combined immunodeficiency mutation in the mouse. *Nature (Lond.).* 301:527.
22. Hardy, R.R. 1986. Purification and coupling of fluorescent proteins for use in flow cytometry. *In* The Handbook of Experimental Immunology, 4th ed. D.M. Weir, L.A. Herzenberg, C.C. Blackwell, and L.A. Herzenberg, editors. Blackwell Scientific Publications, Ltd., Edinburgh. 31.1–31.12.
23. Schulman, M., C.D. Wilde, and G. Kohler. 1978. A better cell line for making hybridomas secreting specific antibodies. *Nature (Lond.).* 276:269.
24. Marcu, K.B., J. Banerji, N.A. Penncavage, R. Lang, and N. Arnheim. 1980. 5′ flanking region of immunoglobulin heavy chain constant region genes displays length heterogeneity in germlines of inbred mouse strains. *Cell.* 22:187.
25. Coleclough, C., R.P. Perry, K. Karjalainen, and M. Weigert. 1981. Aberrant rearrangements contribute significantly to the allelic exclusion of immunoglobulin gene expression. *Nature (Lond.).* 290:372.
26. Strohal, R., A. Helmberg, G. Kroemer, and R. Kofler. 1989. Mouse Vκ gene classification by nucleic acid sequence similarity. *Immunogenetics.* 30:475.
27. Foster, M.H., M. Macdonald, K.J. Barrett, and M.P. Madaio. 1991. V_H gene analysis of spontaneously activated B cells in adult MRL-lpr/lpr mice. *J. Immunol.* 147:1504.
28. Gibson, D.M., G.C. Bosma, and M.J. Bosma. 1989. Limited clonal diversity of serum immunoglobulin in leaky scid mice. *Curr. Top. Microbiol. Immunol.* 152:125.
29. Kearney, J.F., N. Solvasson, R. Stohrer, J. Ma, V. Van Cleave, A. Leheun, G. Fulop, and M. Fried. 1989. Pauciclonal B cell involvement in production of immunoglobulin in scid Ig^+ mice. *Curr. Top. Microbiol. Immunol.* 152:137.
30. Stall, A.M., M.C. Farinas, D.M. Tarlington, P.A. Lalor, L.A. Herzenberg, S. Strober, and L.A. Herzenberg. 1988. Ly-1 B-cell clones similar to human chronic lymphocytic leukemias routinely develop in older normal mice and young autoimmune (New Zealand Black-related) animals. *Proc. Natl. Acad. Sci. USA.* 85:7312.
31. Alt, F.W., G.D. Yancopoulos, T.K. Blackwell, C. Wood, E. Thomas, M. Boss, R. Coffman, N. Rosenberg, S. Tonegawa, and D. Baltimore. 1984. Ordered rearrangement of immunoglobulin heavy chain variable region segments. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:1209.
32. Carmack, C.E., S.A. Camper, J.J. Mackle, W.U. Gerhard, and M.G. Weigert. 1991. Influence of a Vκ8L chain transgene on endogenous rearrangements and the immune response to the HA(SB) determinant of influenza virus. *J. Immunol.* 147:2024.
33. McCormack, W.T., L.W. Tjoelker, L.M. Carlson, B. Petryniak, C.F. Barth, E.H. Humphries, and C.B. Thompson. 1989. Chicken IgL gene rearrangement involves deletion of a circular episome and addition of single nonrandom nucleotides to both coding segments. *Cell.* 56:785.
34. Lafaille, J.J., A. DeCloux, M. Bonneville, Y. Takagaki, and S. Tonegawa. 1989. Junctional sequences of T cell receptor $\gamma\delta$ genes: implications for $\gamma\delta$ T cell lineages and for a novel intermediate of V-D-J joining. *Cell.* 59:859.
35. Feeney, A.J. 1990. Lack of N regions in fetal and neonatal mouse

immunoglobulin V-D-J junctional sequences. *J. Exp. Med.* 172:1377.
36. Ichihara, Y., H. Hayashida, S. Miyazawa, and Y. Kurosawa. 1989. Only D_{FL16}, D_{SP2} and D_{Q52} gene families exist in mouse immunoglobulin heavy chain diversity gene loci, of which D_{FL16} and D_{SP2} originate from the same primordial D_H gene. *Eur. J. Immunol.* 19:1849.
37. Roth, D.B., and J.H. Wilson. 1986. Nonhomologous recombination in mammalian cells: Role for short sequence homologies in the joining reactions. *Mol. Cell. Biol.* 6:4295.
38. Klinman, D.M. 1992. Similarities in B cell repertoire development between autoimmune and aging normal mice. *J. Immunol.* 148:1353.
39. Radic, M.Z., M.A. Mascelli, J. Erickson, H. Shan, and M. Weigert. 1991. Ig H and L chain contributions to autoimmune specificities. *J. Immunol.* 146:176.
40. Nottenburg, C., and I. Weissman. 1981. Cμ gene rearrangement of mouse immunoglobulin genes in normal B cells occurs on both the expressed and nonexpressed chromosomes. *Proc. Natl. Acad. Sci. USA.* 78:484.
41. Coffman, R.L., and I.L. Weissman. 1983. Immunoglobulin gene rearrangement during Pre-B cell differentiation. *Journal of Molecular and Cellular Immunology.* 1:31.
42. Hardy, R.R., C.E. Carmack, S.A. Shinton, J.D. Kemp, and K. Hayakawa. 1991. Resolution and characterization of pro-B and pre-B cell stages in normal mouse bone marrow. *J. Exp. Med.* 173:213.
43. Yancopoulos, G.D., and F.W. Alt. 1986. Regulation of the assembly and expression of variable-region genes. *Annu. Rev. Immunol.* 4:339.
44. Schlissel, M.S., and D. Baltimore. 1989. Activation of immunoglobulin kappa gene rearrangement correlates with induction of germline kappa gene transcription. *Cell.* 58:1001.
45. 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.
46. Benatar, T., L. Tkalec, and M.J.H. Ratcliffe. 1992. Stochastic rearrangement of immunoglobulin variable-region genes in chicken B-cell development. *Proc. Natl. Acad. Sci. USA.* 89:7615.
47. Ehlich, A., S. Schaal, H. Gu, D. Kitamura, W. Muller, and K. Rajewsky. 1993. Immunoglobulin heavy and light chain genes rearrange independently at early stages of B cell development. *Cell.* 72:695.
48. Reth, M., P. Gehrmann, E. Petrac, and P. Wiese. 1986. A novel V_H to V_HDJ_H joining mechanism in heavy-chain-negative (null) pre-B cells results in heavy-chain production. *Nature (Lond.).* 322:840.
49. Kleinfield, R., R.R. Hardy, D. Tarlinton, J. Dangl, L.A. Herzenberg, and M. Weigert. 1986. Recombination between an expressed immunoglobulin heavy-chain gene and a germline variable gene segment in a Ly-1$^+$ B-cell hybridoma. *Nature (Lond.).* 322:843.
50. Meek, K.D., C.A. Hasemann, and J.D. Capra. 1989. Novel rearrangements at the immunoglobulin D locus. *J. Exp. Med.* 170:39.
51. Kienker, L.J., W.A. Kuziel, and P.W. Tucker. 1991. T cell receptor γ and δ gene junctional sequences in SCID mice: excessive P nucleotide insertion. *J. Exp. Med.* 174:769.
52. Carroll, A.M., and M.J. Bosma. 1988. Detection and characterization of functional T cells in mice with severe combined immune deficiency. *Eur. J. Immunol.* 18:1965.
53. Lieber, M.R. 1991. Site-specific recombination in the immune system. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 5:2934.
54. Riggs, J.E., R.S. Stowers, and D.E. Mosier. 1991. Adoptive transfer of neonatal T lymphocytes rescues immunoglobulin production in mice with severe combined immune deficiency. *J. Exp. Med.* 173:265.
55. Reimann, J., A. Rudolphi, and M.H. Claesson. 1991. CD3$^+$ T-cells in severe combined immunodeficiency (scid) mice III. Transferred congenic, self reactive CD4$^+$ T cell clones rescue IgM-producing, scid-derived B cells. *Int. Immunol.* 3:657.
56. Itohara, S., P. Mombaerts, J. Lafaille, J. Iacomini, A. Nelson, A.R. Clarke, M.L. Hooper, A. Farr, and S. Tonegawa. 1993. T cell receptor δ gene mutant mice: independent generation of $\alpha\beta$ T cells and programmed rearrangements of $\gamma\delta$ TCR genes. *Cell.* 72:337.
57. Kurosawa, Y., and S. Tonegawa. 1989. Organization, structure, and assembly of immunoglobulin heavy chain diversity DNA segments. *J. Exp. Med.* 155:201.
58. Gu, H., I. Forster, and K. Rajewsky. 1990. Sequence homologies, N sequence insertion and J_H gene utilization in V_HDJ_H joining: implications for the joining mechanism and the ontogenic timing of Ly1 B cell and B-CLL progenitor generation. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:2133.
59. Feeney, A.J. 1992. Predominance of V_H-D-J_H junctions occurring at sites of short sequence homology results in limited junctional diversity in neonatal antibodies. *J. Immunol.* 149:222.
60. Roth, D.B., and J.H. Wilson. 1988. Illegitimate recombination in mammalian cells. *In* Genetic recombination, R. Kucherlapati and G.R. Smith, editors. American Society of Microbiology, Washington, DC. 621–653.
61. Itohara, S., and S. Tonegawa. 1990. Selection of $\gamma\delta$ T cells with canonical T-cell antigen receptors in fetal thymus. *Proc. Natl. Acad. Sci. USA.* 87:7935.
62. Asarnow, D., T. Goodman, L. LeFrancois, and J.P. Allison. 1989. Distinct antigen receptor repertoires of two classes of murine epithelium-associated T cells. *Nature (Lond.).* 341:60.
63. Itohara, S., A.G. Farr, J.J. Lafaille, M. Bonneville, Y. Takagaki, W. Haas, and S. Tonegawa. 1990. Homing of a γ/δ thymocyte subset with homogeneous T-cell receptors to mucosal epithelia. *Nature (Lond.).* 343:754.
64. Pandey, A., L.W. Tjoelker, and C.B. Thompson. 1993. Restricted immunoglobulin junctional diversity in neonatal B cells results from developmental selection rather than homology-based V(D)J joining. *J. Exp. Med.* 177:329.
65. Asarnow, D.M., D. Cado, and D.H. Raulet. 1993. Selection is not required to produce invariant T-cell receptor γ-gene junctional sequences. *Nature (Lond.).* 362:158.
66. Gerstein, R.M., and M.R. Lieber. 1993. Extent to which homology can constrain coding exon junctional diversity in V(D)J recombination. *Nature (Lond.).* 363:625.
67. Herzenberg, L.A., A.M. Stall, P.A. Lalor, C. Sidman, W.A. Moore, D.R. Parks, and L.A. Herzenberg. 1986. The Ly-1 B cell lineage. *Immunol. Rev.* 93:81.
68. Hayakawa, K., and R.R. Hardy. 1988. Normal, autoimmune, and malignant CD5$^+$ B cells: the Ly-1 B lineage? *Annu. Rev. Immunol.* 6:197.
69. Stall, A.M., S. Adams, L.A. Herzenberg, and A.B. Kantor. 1992. Characteristics and development of the murine B-1b (Ly-1 B sister) cell population. *Ann. NY Acad. Sci.* 651:33.
70. Kantor, A.B. 1991. A new nomenclature for B cells. *Immunol. Today.* 12:338.
71. Hayakawa, K., R.R. Hardy, L.A. Herzenberg, and L.A. Herzenberg. 1985. Progenitors for Ly-1 B cells are distinct from progenitors for other B cells. *J. Exp. Med.* 161:1554.

72. Solvason, N., A. Lehuen, and J.F. Kearney. 1991. An embryonic source of Ly-1 but not conventional B cells. *Int. Immunol.* 3:543.
73. Hayakawa, K., R.R. Hardy, A.M. Stall, L.A. Herzenberg, and L.A. Herzenberg. 1986. Immunoglobulin-bearing B cells reconstitute and maintain the murine Ly-1 B cell lineage. *Eur. J. Immunol.* 16:1313.
74. Hardy, R.R., and K. Hayakawa. 1991. A developmental switch in B lymphopoiesis. *Proc. Natl. Acad. Sci. USA.* 88:11550.
75. Pennell, C.A., L.W. Arnold, G. Haughton, and S.H. Clarke. 1988. Restricted Ig variable region gene expression among Ly-1$^+$ B cell lymphomas. *J. Immunol.* 141:27870.
76. Hardy, R.R., and K. Hayakawa. 1992. Developmental origins, specificities and immunoglobulin gene biases of murine Ly-1 B cells. *Int. Rev. Immunol.* 8:189.
77. Holmberg, D. 1987. High connectivity, natural antibodies preferentially use 7183 and QUPC 52 V_H families. *Eur. J. Immunol.* 17:399.
78. Kaartinen, M., and O. Makela. 1985. Reading of D genes in variable frames as a source of antibody diversity. *Immunol. Today.* 6:3275.
79. Gu, H., D. Kitamura, and K. Rajewsky. 1991. B cell development regulated by gene rearrangement: arrest of maturation by membrane-bound Dμ protein and selection of D_H element reading frames. *Cell.* 65:47.
80. Novick, K.E., T.M. Fasy, M.J. Losman, and M. Monestier. 1992. Polyreactive IgM antibodies generated from autoimmune mice and selected for histone-binding activity. *Int. J. Immunol.* 4:1103.