Preferential Rearrangement of Vκ4 Gene Segments in Pre-B Cell Lines

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Summary

Examination of the in vitro Vκ gene rearrangements of murine adult bone marrow-derived pre-B cell lines reveals that 21 of 25 (84%) cell lines have rearranged a member of the Vκ4 family. In contrast, analysis of two Vκ cDNA libraries prepared from LPS-stimulated adult spleen cells indicates that only 17% of the *Igκ* cDNAs contain sequences belonging to the Vκ4 gene family. Half of the pre-B cell lines examined also share an 8-kbp BamHI reciprocal product (rp). However, these rp do not involve the same Vκ gene, indicating that conserved BamHI sites exist 3′ of some Vκ genes. This rp is also readily detected in DNA from normal adult spleen cells, suggesting that the in vitro rearrangements examined in this study are representative of κ rearrangements that occur in vivo. We suggest that, unlike the diverse Vκ repertoire expressed by mature B cells, the germline Vκ segments involved in initial rearrangements of the *Igκ* locus are highly restricted, and that an initial Vκ4 rearrangement is probably followed by other, more random recombination events.

The development of the B cell repertoire relies on the rearrangement and expression of both heavy and light chain Ig variable region genes. These well-studied rearrangement events are the basis of the enormous diversity of antigen binding sites found among antibodies. Defining the numerous genetic and cellular processes and interactions that influence the somatic evolution of the primary B cell repertoire is fundamental to a full understanding of immunocompetence and the apparently programmed fashion in which the adult primary repertoire is formed (1).

Several groups have investigated the expression of Ig V region genes in adult and early B cell populations. It has been observed that B lineage cells derived from mouse fetal liver preferentially rearrange D-proximal V_H segments (2–4), and studies of human leukemic and fetal B cells have also demonstrated a nonrandom usage of V_H gene families (5–7). In contrast, the pattern of V_H gene expression among normal adult murine splenic B cells is a more faithful reflection of the germline library of V_H segments (8–11).

To gain insight into the mechanism and function of preferential V gene rearrangements, it is important to determine whether initial rearrangements on other Ig loci are also restricted to particular sets of V genes. Recent advances in the classification of mouse Vκ gene families (12, 13) have permitted several groups to begin to examine Vκ gene utilization in systems analogous to those used to study V_H genes (14–16).

The Ig κ light chain locus of the mouse is comprised of 100–300 variable and 4 functional joining region (Jκ) gene segments (reviewed in reference 17). By amino acid sequence criteria there are 24 known groups (18), and hybridization with Vκ probes has defined at least 16 Vκ gene families, some of which encompass more than one of the previously described Vκ groups (12, 13).

In this study we have examined the Vκ genes involved in early rearrangement events that occur in A-MuLV-transformed pre-B cell lines during in vitro propagation. We show that, in contrast to the primary Vκ splenic B cell repertoire, most (21/25) cell lines have rearranged at least one member of the Vκ4 family. We suggest that initial *Igκ* locus V gene rearrangements are highly restricted and that the B cell repertoire is likely to be significantly influenced by subsequent recombination events.

Materials and Methods

Cell Lines. Cell lines described in this study are A-MuLV-transformed pre-B cell lines derived from adult bone marrow. A total of 25 lines were analyzed, 9 of which are either primary or secondary subclones of the BALB/c cell line BM18-4. BM18-4 was kindly provided by Dr. Naomi Rosenberg (Tufts Medical School), and has been previously shown to rearrange its κ loci during propagation in vitro (19, 20). BM18-4 was cloned by plating the cells at a density of 0.3 cells/well in 96-well plates. Primary subclones K3, K6, K10, and K23 were further subcloned to obtain the secondary subclones used in this study. All plates contained fewer than 17 positive wells after 10 d. Cell lines were grown as described previously (21). The remaining 16 lines were each independently derived; 8 were obtained from a panel of CXXB cell lines of

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(BALB/c.xid × C57BL/10)F_1 origin, described in detail elsewhere (21), and 8 cell lines were derived from (BALB/c × CBA)F_1 (CXCB) mice, and were made in the same manner as the CXXB lines.

Probes. The hybridization probes used in this study were agarose gel-purified DNA fragments. The Cκ probe (Fig. 3 *B*) is a 3.8-kbp XbaI/BamHI fragment isolated from pECκ (22), kindly provided by Dr. Robert Perry (Fox Chase Cancer Center, Philadelphia, PA). The pRI probe is a 900-bp EcoRI fragment from pRI (22), kindly provided by Dr. Brian Van Ness (University of Minnesota, Minneapolis, MN). The Vκ4 probe is a 178-bp BamHI/AluI fragment, cloned from cell line K3-2, containing codons 42–95 of the V region and 20 bp of the 5′ end of Jκ5. The Vκ4 probe does not cross-hybridize with other Vκ families under the hybridization conditions used in this study. To verify probe specificity, DNA samples from 200 $C\kappa^+$ phage, taken from two $C\kappa^+$ cDNA libraries, were screened with 12 Vκ family probes (including the Vκ4 probe) using Southern blot hybridization conditions (see below). The Vκ4 probe did not demonstrate any crossreactivity with any of the other Vκ families tested: Vκ1, Vκ8, Vκ9, Vκ10, Vκ11, Vκ19, Vκ21, Vκ22, Vκ23, Vκ24, Vκ28. In addition, all of these Vκ family probes, including the Vκ4 probe, gave the expected restriction fragment patterns on Southern blots based on published data (12, 13).

Southern Blots. Southern blotting and hybridizations were performed as described previously (21). Briefly, DNA (10 μg/lane) was digested to completion, fractionated through a 0.7% agarose gel, transferred to a nylon membrane, and hybridized with the appropriate DNA probe. Final washing of the blots was in 0.2× SSC at 68°C for 30 min.

Northern Blots. Total RNAs were fractionated through 1% agarose gels containing 2.2 M formaldehyde and transferred to nylon membranes. Blots were hybridized in 5× SSC, 5× Denhardt's solution, 50 mM sodium phosphate (pH 6.5), 250 μg/ml sonicated salmon sperm DNA, and 50% deionized formamide. After overnight hybridization at 42°C with ^{32}P-labeled probe, blots were washed twice at 50°C in 1× SSC, 0.1% SDS for 15 min/wash.

Cloning and Sequencing. The 4.3-kbp BamHI fragment from cell line K3-2, and the 1.5-kbp BglII fragment from cell line K6-22 were cloned into the Charon 27 λ vector and recombinant phage were identified using the Vκ4 probe. The 8-kbp reciprocal product (rp)[1] from cell line K6-9 was also cloned into Charon 27 and recombinant phage were identified using the pRI probe. Vκ4 and rp-containing fragments were subcloned into M13 mp10 and M13 mp19 phage. The sequences of both strands were determined using protocols and a modified form of T7 DNA polymerase (Sequenase) obtained from U.S. Biochemical Corporation (Cleveland, OH).

Vκ cDNA Libraries. The cDNA libraries were made as described in detail elsewhere (11). Briefly, poly(A)$^+$ mRNA was obtained from adult BALB/c spleen cells cultured for 3 d with 50 μg/ml LPS. RNAs were primed with a Cκ-specific oligonucleotide. Double-stranded cDNA was synthesized and cloned into the bacteriophage vector λgt10. Phage were plated with *C600 Hfl* indicator bacteria and lifted onto nitrocellulose filters for subsequent hybridizations. The Cκ oligonucleotide is a 20-mer consisting of codons 68–74: 5′ GGTCAACGTGAGGGTGCTGC 3′.

Results and Discussion

A 4.3-kbp Igκ Rearrangement Is Frequently Observed in A-MulV-transformed Pre-B Cell Lines. 25 A-MuLV transformed cell lines derived from adult bone marrow were chosen for study based on the detection of a rearranged *Igκ* locus, as demonstrated by Southern blot hybridization of a Cκ probe to BamHI-digested DNA (Fig. 1). Six cell lines (K3-2, N4, N15, N20, M46, E5) have multiple rearranged bands of varying intensities, suggesting active rearrangement, while the remainder appear to have stably rearranged *Igκ* loci.

A striking feature of the *Igκ* rearrangements in these cell lines (Fig. 1, Table 1) is the frequent occurrence of a Cκ-hybridizing 4.3-kbp BamHI fragment (14/25 cell lines). To determine whether these 4.3-kbp BamHI fragments contain independent rearrangements of the same or related germline Vκ gene segments, the 4.3-kbp fragment was cloned from cell line K3-2 (Fig. 1). The 5′ region (490 bp) of this fragment was further subcloned and sequenced. A search against the Beckman Microgenie Sequence Analysis Program (Beckman Instruments, Inc., Palo Alto, CA) revealed that this region contained a member of the Vκ4 family joined in frame to Jκ5. The partial sequence of this Vκ4 gene (codons 42–95) is identical to the published germline H4 Vκ4 gene (23).

Southern Blot Analysis Reveals a Preferential Rearrangement of the Vκ4 Family. Using the cloned Vκ4 gene from cell line K3-2 as a hybridization probe for Southern blots of BglII-digested DNA (Fig. 2), the panel of cell lines was examined to determine if identical Vκ4 rearrangements had occurred in other cell lines, and to determine the frequency of rearrangements involving Vκ4 family members. This analysis revealed that 72% (18/25) of the lines had rearranged a member of the Vκ4 family (Fig. 2, Table 1). In addition, a large number of the cell lines share a Vκ4-hybridizing nongermline BglII fragment of 1.5 kbp (Fig. 2, Table 1). These 1.5-kbp fragments may reflect identical Vκ4-Jκ joins, since they contain Jκ sequence as well (data not shown), or they may represent reciprocal products resulting from inversional Vκ-Jκ rearrangements (24–26). Inversion of some Vκ4 gene segments has been described previously (27), and inversional joins can result in rp containing adjacent, nonjoined Vκ and Jκ sequences in nongermline contexts. Therefore, to ascertain whether these fragments contain a reciprocal product or a Vκ-Jκ join, the 1.5-kbp BglII fragment from cell line K6-22 (Fig. 2) was cloned and partially sequenced. An AluI fragment was found to contain codons 26–95 of a Vκ gene identical in sequence to the germline R9 Vκ4 gene (23), joined in frame to Jκ5. This cloned Vκ4 gene also shows a 91% nucleotide sequence identity with the rearranged Vκ4 gene cloned from cell line K3-2 (discussed above). Thus, the rearranged Vκ4 genes in cell lines K3-2 and K6-22 derived from distinct germline genes, indicating that more than one Vκ4 gene was involved in the rearrangements observed.

As demonstrated in Fig. 2, one cell line (M46) has four detectable BglII Vκ4 rearranged fragments. This feature may reflect the fact that this cell line was actively rearranging its kappa loci when the DNA was prepared. Another formal possibility is that some of these Vκ4-containing fragments represent rp of *Igκ* rearrangement. However, the excellent correlation between detectable rearranged Vκ4 fragments and Vκ4 RNA expression (Table 1 and discussed below), and the limited cloning and sequencing data presented here, suggest

[1] *Abbreviations used in this paper:* rp, reciprocal product.

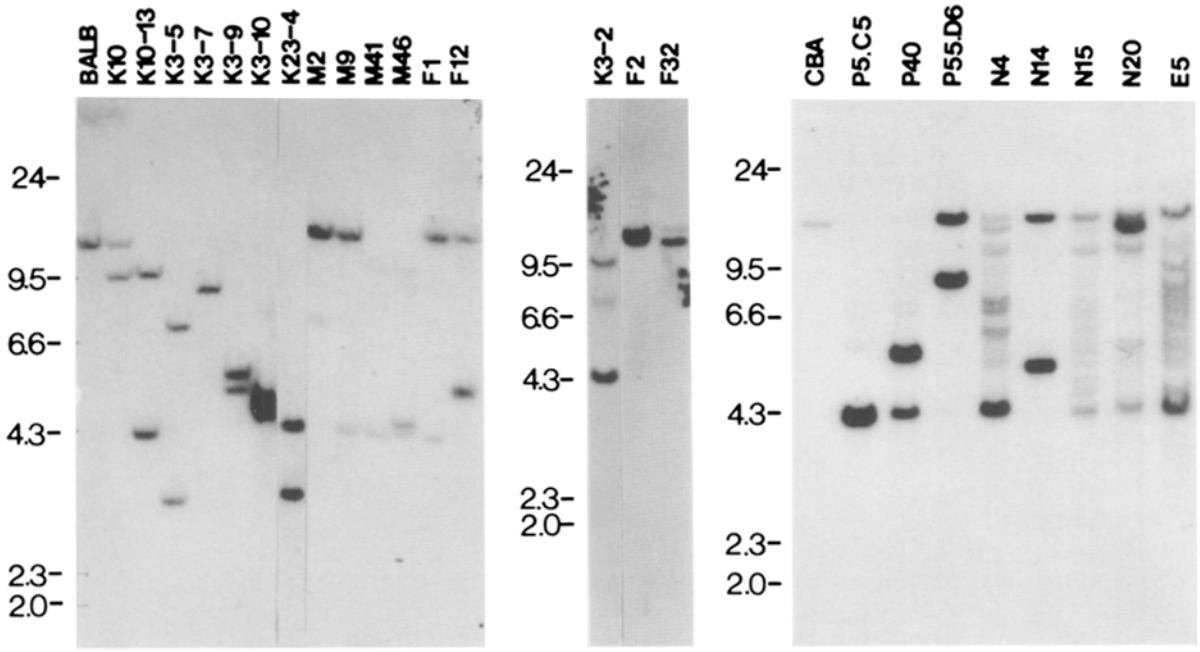

Figure 1. *Igκ* locus rearrangement in A-MuLV-transformed pre-B cell lines. Southern blots of BamHI-digested DNA from A-MuLV-transformed cell lines were hybridized with the Cκ probe to screen for *Igκ* locus recombination. All lines are clonal, although not all have stably rearranged their *Igκ* loci. The submolar bands seen in certain lanes are due to the continued rearrangement of the *Igκ* loci in these cell lines while in culture. The P5.C5 cell line is a subclone of the P5 cell line listed in Fig. 2 and Table I. The P5 parental line also exhibits a 4.3-kbp rearrangement. The 4.3-kbp fragment of cell line K3-10 ran slowly due to overloading of the gel. Additional blots show that this fragment is indistinguishable in size from the other 4.3-kbp fragments. Subclone K6-22 is not shown. The germline BamHI Cκ fragment migrates at 12.7 kb.

that all or most of the Vκ4 rearrangements observed are Vκ to Jκ joins and not rp of rearrangement.

Blackwell et al. (28) have also recently suggested, and provided evidence for, the early and preferential rearrangement of the Vκ4 family. Their recent investigation of *Igκ* locus rearrangements in A-MuLV transformed pre-B cell lines from C.B.17 *scid* mice showed that in the five signal joins (reciprocal products of inversional rearrangements) examined, the sequences immediately 3′ of the Vκ nonamers had >80% nucleotide sequence identity with the 3′ flank of VκL8, a member of the Vκ4 family. (Blackwell et al. refer to VκL8 as a Vκ5 family member. Due to a high degree of nucleotide sequence identity the Vκ4 and Vκ5 families have previously been reclassified as one family [12]). In addition, a recent investigation (16) using hybridomas from fetal liver and day 1 neonatal liver, and subclones from an A-MuLV-transformed pre-B cell line, 18–81, indicated preferential usage of Vκ4 as well, although with a lower frequency (32%) than was observed in our study. Although the basis for the lower frequency of Vκ4 rearrangement by Lawler et al. is not known, the cell lines examined by them included hybridomas generated from fetal and neonatal tissues, sources of B cells not examined in our study, and which may represent B cells at a more mature stage of development. It is possible, therefore, that they may have missed some of the earliest recombination events. Also, the remaining cell lines all derived from one parental cell line and might not accurately reflect the repertoire of early κ locus rearrangements.

To determine if another family might also be highly represented in our panel of cell lines, the lines were screened for Vκ1 rearrangement. Like Vκ4, the Vκ1 family is fairly complex with 12 fragments resolved on Southern blots of BamHI-digested DNA (the Vκ4 probe hybridizes to 16 BamHI fragments). In contrast to the high frequency of Vκ4 rearrangements, of 25 cell lines only 3 exhibited Vκ1 rearrangements (K3-7, K3-9, M9). These three lines were also positive for Vκ4 rearrangement.

Analysis of RNA From A-MuLV-transformed Cell Lines also Indicates a Preferential Usage of the Vκ4 Family. Since the Vκ4 probe detects 17 BglII restriction fragments on a Southern blot, it was possible that some BglII fragments were undetectable because of comigration with germline restriction fragments bearing Vκ4 genes. Therefore, Northern blot analysis was performed using total RNAs from the panel of cell lines (data not shown), and it was revealed that three of the lines without a detectable Vκ4 gene rearrangement express mature Vκ4 mRNAs (K10, N4, E5). Furthermore, 11 of the 18 Vκ4-rearranged cell lines also express mature Vκ4 mRNA transcripts (Table 1), verifying the use of a Vκ4 family member by these lines. Six of the seven Vκ4-rearranged, Vκ4 mRNA-negative cell lines (except one, K3-9), were negative for expression of the κ locus (Table 1), as determined by Northern blots hybridized with the Cκ probe (data not shown). Together, the Southern and Northern data show that 84% (21/25) of the cell lines have rearranged at least one Vκ4 gene.

Although the present data clearly establish a preferential usage of the Vκ4 family, the possibility of another family also being preferentially rearranged cannot be excluded. How-

Table 1. *Summary of Igκ Locus Rearrangements and RNA Expression in A-MuLV-transformed Cell Lines*

Cell line*	Detectable Vκ4 rearrangements‡	Cκ mRNA§	Vκ4 mRNA‖	4.3-kbp BamHI Cκ fragment¶	1.5-kbp BglII Vκ4 fragment**	8-kbp BamHI rp‡‡
K10	−	+	+	−	−	−
K10-13	+ (1)	+	+	+	−	−
K3-2	+ (2)	+	+	+	+	+
K3-5	+ (1)	+	+	−	+	−
K3-7	+ (1)	+	+	−	−	+
K3-9	+ (1)	+	−	−	−	+
K3-10	+ (1)	+	+	+	+	−
K6-22	+ (1)	+	+	−	+	+
K23-4	+ (1)	+	+	+	+	+
M2	+ (1)	−	−	−	+	−
M9	+ (1)	−	−	+	−	−
M41	+ (2)	−	−	+	−	+
M46	+ (4)	+	+	+	+	+
F1	−	−	−	+	−	−
F2	+ (1)	−	−	−	−	−
F12	+ (1)	−	−	−	−	−
F32	+ (2)	−	−	−	−	−
P5	+ (1)	+	+	+	+	−
P40	+ (1)	+	+	+	+	−
N4	−	+	+	+	−	+
N14	−	−	−	−	−	+
N15	−	+	−	+	−	+
N20	+ (1)	+	+	+	+	+
P55.D6	−	−	−	−	−	−
E5	−	+	+	+	−	+
Totals§§	18 (24)	16	14	14	10	12

* Groups of cell lines prefixed by different capital letters were derived from independent transformations of adult bone marrow cells.
‡ The number of rearranged Vκ4 gene segments was determined by Southern blot analysis of BglII-digested DNA using the Vκ4 probe.
§ The expression of full-length Cκ transcripts was determined by Northern blot analysis using the Cκ probe.
‖ The expression of full-length κ transcripts of the Vκ4 family was determined by Northern blot analysis using the Vκ4 probe.
¶ The presence of a 4.3-kbp BamHI fragment containing Cκ sequence was determined by Southern blot analysis using the Cκ probe.
** The presence of a 1.5 kbp BglII fragment was determined by Southern blot analysis using the Vκ4 probe.
‡‡ Reciprocal products, resulting from κ locus rearrangements, were identified on Southern blots of BamHI-digested DNA hybridized with a probe containing sequences 5′ of germline Jκl (pRl).
§§ Number of positive cell lines.

ever, Northern blot analyses for families Vκ1, Vκ8, Vκ19, Vκ21, Vκ22, and Vκ28 (data not shown), do not indicate a biased utilization of any of these families. We conclude, therefore, that the preferential rearrangement of Vκ4 gene segments is a general feature of A-MuLV-transformed pre-B cell lines and is not restricted to one particular pre-B cell line, nor is it a bias of a particular transformation experiment (Table 1).

The Vκ4 Family Is Not Overexpressed in the Primary Adult Vκ Repertoire. Given the remarkably high frequency of Vκ4 gene rearrangements in our cell lines, we asked whether this preferential utilization of the Vκ4 family influences the primary adult antibody repertoire. To address this, a Vκ cDNA phage library was constructed using poly(A)$^+$ mRNA from adult BALB/c spleen cells cultured for 3 d with LPS. The primary library contained 340,000 recombinant phage, and it was determined by plaque lift hybridization that 70% of the cDNAs were Cκ$^+$. >6,000 Cκ$^+$ phage were screened using the Vκ4 probe, and it was calculated that 17% of the cDNAs contain a Vκ4 gene. Data from a second independent cDNA library confirmed these results. A recent study

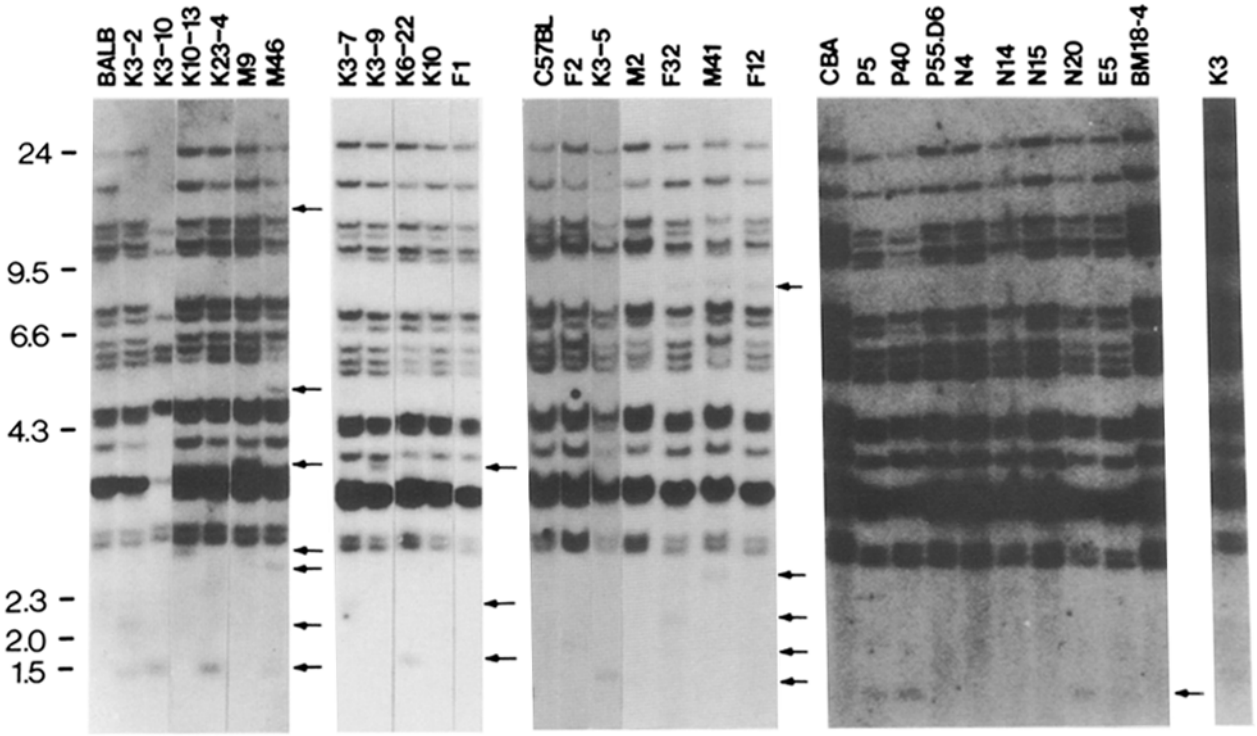

Figure 2. Vκ4 rearrangement in A-MuLV-transformed pre-B cell lines. Southern blots of BglII-digested DNA from A-MuLV-transformed cell lines were hybridized with the Vκ4 probe. Arrows indicate Vκ4 rearrangements.

by Teale and Morris (15) obtained similar results using in situ hybridization for both adult and neonatal Vκ4 family usage, while Kaushik et al. (14) reported an even lower frequency for Vκ4 usage using a B cell colony blot assay. Therefore, in contrast to our finding that 84% of the pre-B cell lines examined have a rearranged Vκ4 gene on at least one chromosome, the frequency of Vκ4 expression in normal splenic B cells appears more consistent with the relative proportion of Vκ4 germline genes (12, 13).

An 8-kbp BamHI Reciprocal Product Is Frequently Found in Both A-MuLV-transformed Pre-B Cell Lines and Adult Splenic B Cells. Given the apparently nonrandom use of the Vκ4 family, a family with members known to rearrange by inversion resulting in rp, we asked whether our panel of cell lines also share similar rp. All of the cell lines have undergone at least one inversional rearrangement as determined by the presence of nongermline BamHI fragments (reciprocal products) that hybridize to pRI (Fig. 3 *B*), a probe containing sequence 5′ of the Jκ region (data not shown). Furthermore, nearly half of the lines contained a reciprocal product located on BamHI fragments of the same size (8 kbp, Table 1). The presence of an rp was confirmed by cloning and sequencing a portion of this 8-kbp fragment from cell line K6-9. This rp was found to contain a back-to-back joining of the heptamer-nonamer signal sequences from Jκ1 and a member of the Vκ 12,13 family (Fig. 4), as determined by a 93% sequence identity with the published consensus sequence of K2 (29), a germline Vκ 12,13 gene. This suggests that an additional rearrangement involving a Vκ family other than Vκ4 has occurred, and it is also, to our knowledge, the first evidence that a Vκ 12,13 family member can rearrange by inversion.

If the frequent presence of 8-kbp BamHI rps in our cell lines is indicative of rearrangements that occur in vivo, then an 8-kbp BamHI rp should also be detected in normal tissue. Indeed, Southern blots of neonatal and adult spleen DNA revealed an 8-kbp BamHI fragment in adult spleen but not neonatal spleen or liver (Fig. 3 *A*). The absence of an 8-kbp rp in neonatal liver and spleen was most likely due to the small number of B cells present in neonatal tissues. The rearranged BamHI fragment detected in normal spleen was indistinguishable in size from the 8-kbp fragment frequently observed in our cell lines, for example K23-3 in Fig. 3 *A*. By densitometry, ~27% of the *Igκ* loci from IgM-expressing spleen cells exhibited this 8-kbp rp (data not shown). Although we have no evidence regarding the nature of the 8-kbp rp in normal B cells, these data support the possibility that the in vitro rearrangements observed in pre-B cell lines reflect those occurring in vivo.

To determine if all of the 8-kbp BamHI fragments contain the identical rp, a 221-bp HaeIII/XbaI fragment 3′ of the Vκ12,13 signal sequence (Fig. 4) was used to screen the panel of cell lines. Only one cell line (K6-22) other than K6-9, the cell line from which the rp was cloned, gave a positive signal in the 8-kbp region (data not shown). Therefore, the other 8-kbp rp are products of rearrangement events involving a Vκ gene or genes different than in cell line K6-9.

Two other groups have also found 8-kbp BamHI rp-containing fragments, neither of which, however, involved

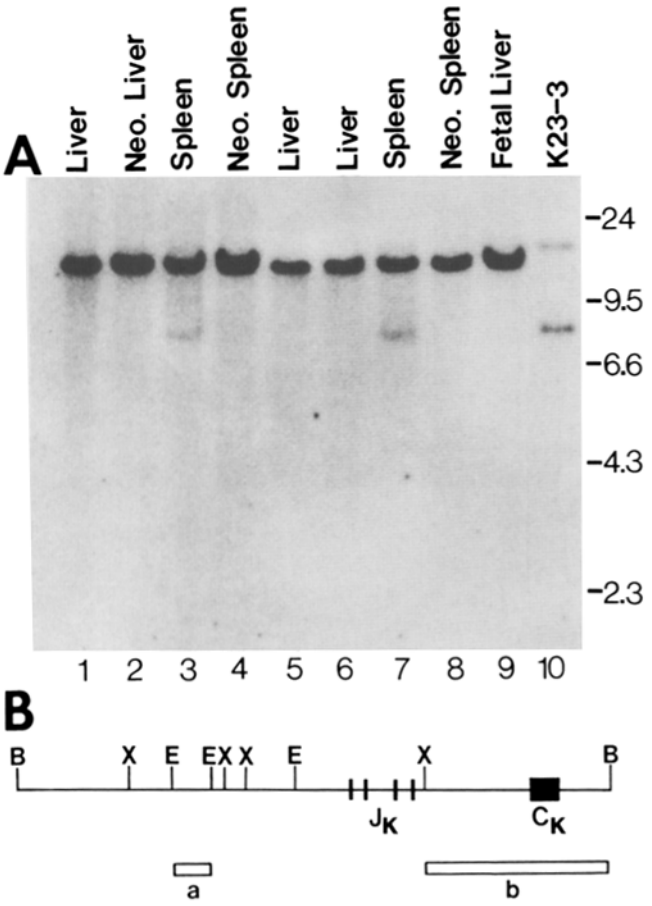

Figure 3. (*A*) An *Igκ* locus rp in normal tissue is detected on a Southern blot of BamHI-digested DNA hybridized with the pRI probe. The germline BamHI fragment migrates at 12.7 kb, and the reciprocal product appears at ~8.0 kbp. DNA from lanes *1–4* and *10* are of BALB/c origin, lanes *5–8* of (BALB/c × CBA)F_1 origin, and lane *9* of CBA origin. BALB/c neonatal liver and spleen, in lanes *2* and *4*, respectively, were taken on day 1. Neonatal spleen in lane *8* was taken on day 8, and the fetal liver in lane *9* was taken on day 17 of gestation. (*B*) Shown are probes used in the analysis of reciprocal products, pRI (*a*), and in the analysis of rearrangement at the *Igκ* locus, Cκ (*b*). For scale, probe *a* is 900 bp in length.

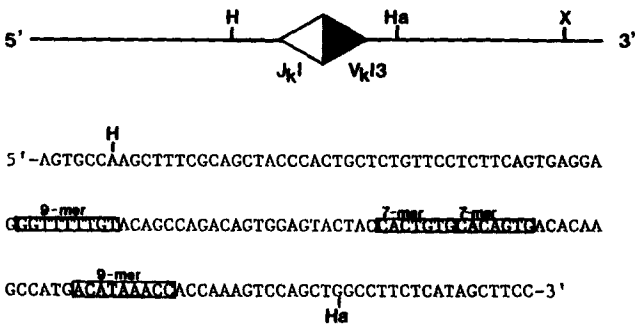

Figure 4. Diagram of the rp cloned from cell line K6-9, and its sequence. Over 400 bases were sequenced beginning at the 5′ HindIII (H) site. In the accompanying sequence the heptamers and nonamers are boxed. Ha, HaeIII; X, XbaI.

a Vκ12,13 gene. Rather, one contained the 3′ flank of a Vκ10 gene (27), and the other contained the 3′ flank of a Vκ8 gene (Clarke, S., personal communication). Together, these findings suggest that Vκ genes from at least four families contain conserved BamHI sites 3′ of their coding regions, resulting in 8-kbp BamHI rps upon inversional recombination. Whether these families include genes that are frequently involved in *Igκ* locus rearrangements, secondary rearrangements in particular (discussed below), awaits further investigation.

Functional Significance and Mechanism for the Preferential Usage of the Vκ4 Family. The phenomenon of the preferential usage of V_H genes proximal to the D region, originally observed in A-MuLV-transformed cell lines, has recently been demonstrated for normal fetal tissue (4). Thus, it is possible that the preferential rearrangement of Vκ4 genes will also be found to exist in vivo. What functional significance might a high frequency of Vκ4 gene rearrangements have in developing cells? One possibility extends the suggestion by Shapiro and Weigert (27) that inversional rearrangements might provide a means by which more distal Vκ gene segments can be repositioned closer to Jκ segments, thereby increasing their probability for recombination. This speculation is consistent with the findings that multiple, sequential Vκ rearrangements can occur on a single chromosome (26, 27, 30). Considering the size of the Vκ4 family, its central position within the Vκ region, and the ability of at least some Vκ4 genes to rearrange by inversion, the Vκ4 family may play a special role in bringing Jκ segments and distal Vκ genes together for recombination. The Vκ4 family is not, however, the only family that could serve this function. Therefore, an additional factor, such as chromatin structure or accessibility (31) must be involved in creating this bias for Vκ4.

Although the data presented support the idea of Vκ4 recombination being used as a vehicle for subsequent random rearrangements of distal Vκ families, it is not known that Vκ4 recombination is actually occurring first in the process. However, since both Vκ4 rearrangements that were cloned from cell lines in this report were in-frame rearrangements involving Jκ5, and since at least some Vκ4 genes rearrange by inversion, it is possible that an inversional recombination of a Vκ4 gene to Jκ5 (or Jκ2-Jκ4) is the primary event that occurs. Because this is an inversional event, Jκ1-Jκ4 are displaced on the chromosome rather than lost. Therefore, Jκ1-Jκ4 would remain available as substrates for recombination with proximal Vκ genes resulting in subsequent rearrangements; however, these rearrangements would be nonfunctional given their distance from Cκ. In fact, as mentioned previously, evidence exists that indicates that more than one rearrangement can occur on a single allele, and there are documented cases of plasmacytomas that contain double recombination products, products that result from more than one rearrangement occurring on the same allele (26). These subsequent rearrangements could result in the deletion of the initial rp generated from the primary recombination event as well as subsequent rps and VJ rearrangements. This has already been proposed as an explanation for plasmacytomas that lack reciprocity between the rp identified and the expressed Vκ gene (26, 27).

The mechanism behind the preferential usage of the Vκ4 family remains unclear. Proximity to J, which has been suggested to play a role in preferential V_H gene usage in A-MuLV–transformed pre-B cells (2, 3), does not appear to be correlated with preferential Vκ4 rearrangement. Current data on the organization of the *Igκ* locus (12, 13) position several Vκ families between Vκ4 and Jκ. However, interspersion of Vκ and V_H gene families has been well documented (21, 32, 33), and it is possible that a subset of the Vκ4 family may be more proximal to Jκ and that these are the rearranged genes being observed. Furthermore, the relatively large size of the Vκ4 family may contribute to its frequent rearrangement, but it is unlikely the sole contributing factor since Vκ4 genes probably represent no more than 10–20% of the Vκ segments in the *Igκ* locus (12, 13). An intriguing possibility, recently suggested by Tutter and Riblet (34) for the *Igh*-V locus, is that certain V-gene sequences are evolutionally conserved to maintain noncoding functions; for example, recombination. Thus, Vκ4 sequences may intrinsically be highly recombinogenic.

As with the preferential rearrangement of particular V_H genes in mouse (2, 3) and human (5–7), the functional significance and mechanism of preferential Vκ4 rearrangements is unknown. Understanding those factors responsible for the biased expression of a particular V gene is especially important when considering certain disease states, such as chronic lymphocytic leukemia in which there is a high expression of one particular κ light chain gene (35, 36). In addition, there remains the question of how the primary B cell Vκ and V_H repertoires become more representative of the inherited V gene segments, thereby allowing the considerable germline complexity of these loci to be utilized. Specifically, it is unclear what prevents the Vκ4 family from dominating the adult splenic Vκ repertoire. One possibility is that Vκ4-rearranged pre-B cells are unable to escape from the bone marrow into the periphery, or once in the periphery the cells may undergo gene replacement. When considering the antibody pool, it is conceivable that Vκ4 protein exhibits poor V_H chain pairing, thereby thwarting an overrepresentation of Vκ4-bearing antibody. Undoubtedly, the regulatory process responsible for the formation of the Vκ gene repertoire will involve numerous components at various stages of development.

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