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Early rearrangements of genes encoding murine immunoglobulin κ chains, unlike genes encoding heavy chains, use variable gene segments dispersed throughout the locus

(B-cell/ontogeny/chromosomal map/sequence)

ANN M. LAWLER*, JOHN F. KEARNEY†, MICHAEL KUEHL‡, AND PATRICIA J. GEARHART*§

*Department of Biochemistry, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD 21205; †Department of Microbiology, University of Alabama, Birmingham, AL 35294; and ‡National Cancer Institute–Naval Medical Oncology Branch, National Naval Medical Center, Bethesda, MD 20814

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ABSTRACT **Immunoglobulin heavy-chain variable region (V_H) gene segments located closest to the joining (J_H) gene segments are preferentially rearranged during ontogeny, indicating that chromosomal position influences the frequency of rearrangement. In addition, certain V_H gene segments are repeatedly rearranged, suggesting that the DNA sequence or structure surrounding these segments may increase the probability of rearrangement. To determine whether there is similar biased rearrangement of κ variable ($V_κ$) gene segments, 25 rearrangements were sequenced from murine fetal and neonatal B-cell hybridomas and from subclones of a pre-B cell line that rearranged $V_κ$ genes during *in vitro* culture. Four gene segments were isolated twice and one gene segment was isolated three times, suggesting that the process that targets individual variable gene segments for repeated rearrangement operates on both the V_H and $V_κ$ loci. Based on a current map of the $V_κ$ locus, the rearranged gene segments belong to nine families that are dispersed throughout the locus. Thus, in these cell types, $V_κ$ rearrangements use germ-line gene segments located across the entire locus, whereas the corresponding V_H rearrangements use gene segments proximal to the J_H gene segments. Heterogeneity of $V_κ$ rearrangements would add diversity to the biased pool of V_H rearrangements, producing a broad repertoire of antibodies early in development.**

Immunoglobulins are encoded by multigene families in which rearrangement activates their expression. In mice, several hundred heavy-chain variable region (V_H) and κ-chain variable region ($V_κ$) gene segments are grouped into 11 V_H families and 18 $V_κ$ families. A family contains gene segments with >80% nucleotide homology and varies in size from 2 to >100 elements. Preliminary mapping data suggests that the families are dispersed over ≈500–2400 kilobase (kb) pairs for each locus (1, 2). The organization of gene segments may play a significant role in controlling their rearrangement.

The rearrangement patterns of V_H gene segments have been extensively analyzed early in development. A number of investigators (3–8) have examined the pattern in fetal and neonatal pre-B cells from BALB/c mice and found overrepresentation of the heavy-chain joining region (J_H) gene segment proximal families, V_H7183 and V_HQ52. The biased pattern of rearrangements suggests that the V_H7183 and V_HQ52 families are in a region of chromatin that is more accessible to rearrangement than that of the other families. Within this region, processes appear to be acting on a more discrete level that target rearrangement to individual V_H gene segments. Several studies have observed repeated rearrangements of specific V_H gene segments: V_H*81X* of the V_H7183 family (3, 5), V_H*Ox2* of the V_HQ52 family (5), and four gene segments in human (9). The repeated rearrangements occurred in pre-B cells, in the absence of light-chain expression, and, thus, were not the result of selection by endogenous antigen. Repeated rearrangements may be caused by variations in local chromatin structure making the loci more accessible for recombination or by differences in DNA sequences that give genes higher affinity for recombination enzymes. In this study, we examined the pattern of $V_κ$ rearrangements¶ during ontogeny to determine whether similar processes are also operating on the κ locus.

MATERIALS AND METHODS

Cell Lines. Hybridomas were made by fusing lymphocytes from livers of day-16 to -19 fetal and 1-day-old BALB/c mice with P3-X63Ag8.653 myeloma cells and were screened for heavy- and κ-chain expression by an ELISA assay. The V_H families expressed by these cell lines were identified on RNA dot blots using the V_H7183, V_HQ52, and V_HS107 probes identified in ref. 5, and a V_H3660 probe consisting of a 635-base-pair (bp) *Eco*RI–*Xba* I fragment from the V_H*SB32* gene (10). BFL14 is a fetal liver cell line transformed with Abelson virus. The 18-81 progenitor cell line was generated from adult bone-marrow cells of a BALB/c mouse by *in vitro* transformation with Abelson murine leukemia virus and is representative of a pre-B cell in that it produces cytoplasmic μ heavy chain but no κ light chain (11). Subclones of 18-81 were identified that had spontaneously rearranged $V_κ$ gene segments during culture. Several 18-81 subclones contained more than one rearrangement due to continued rearrangement after subcloning.

Cloning and Sequencing. $V_κ$ rearrangements were identified on Southern blots of genomic DNA prepared from each cell line and digested with *Hin*dIII or *Bam*HI. The probe was a 1.0-kb *Xba* I–*Hin*dIII fragment from the intervening sequence between κ-chain joining region ($J_κ$) and κ-chain constant region ($C_κ$) gene segments and was labeled with [^{32}P]dATP using random hexamers. The gene segments were cloned into λ Charon 28 from *Hin*dIII-digested genomic DNA that was size-selected on 1% agarose gels. The resulting libraries were screened with the probe, and $V_κ$ rearrangements were subcloned into the plasmid vector, pTZ19R (Pharmacia), and sequenced using 4 primers homologous to 20 nucleotides at the 3′ end of each $J_κ$ gene segment.

Abbreviations: V_H and J_H, heavy-chain variable and joining gene segments; $V_κ$ and $J_κ$, κ light-chain variable and joining gene segments; *D* and *C*, diversity- and constant-region gene segments, respectively.

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§To whom reprint requests should be addressed.

¶The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M25996–M26003).

RESULTS

Two types of cell populations from BALB/c mice were studied: (*i*) hybridoma cell lines generated from fetal and neonatal liver B cells that rearranged V_κ gene segments *in vivo*, and (*ii*) subclones of a cell line derived from a bone-marrow pre-B cell that rearranged V_κ gene segments *in vitro*. The hybridomas were chosen to allow a direct comparison between the patterns of V_κ and V_H rearrangements in a population of cells known to frequently rearrange J_H proximal V_H gene segments (3, 4). Similarly, V_H usage in this particular group of hybridomas was biased in that 5 out of 12 lines used the J_H-proximal families, V_H7183 and V_HQ52. Specifically, as determined by RNA hybridization (A.M.L., unpublished results) and DNA sequencing of BFL14 (5), four lines used V_H7183: 15-56-1, 134-1, BC2-5-12, and BFL14; two lines used V_HQ52: GB3-1 and BFL14; one line used V_HS107: ID3-2; two lines used V_H3660: GB3-1 and 4-1; and four lines were not identified. Out of 12 V_κ rearrangements, 10 appeared to be productive by sequencing and could encode light chains expressed as surface immunoglobulin (Fig. 1). Therefore, the hybridomas likely reflect the V_κ repertoire found early in development; this repertoire may have undergone positive or negative selection by interaction of surface immunoglobulin with endogenous antigens within the animal.

We also examined 13 V_κ rearrangements in 6 subclones of an Abelson-transformed pre-B cell line, 18-81, that rearranged V_κ gene segments in culture and would not be influenced by selective pressures within an animal (Fig. 1). Because the pattern of V_H rearrangements generated in culture is comparable to that seen *in vivo* (3, 20), the 18-81 subclones are likely to represent the V_κ rearrangement pattern in the animal.

To detect repeated rearrangements of individual V_κ gene segments, each one was cloned and sequenced. Gene segments from nine V_κ families were identified (Figs. 1 and 2). No somatic mutations were found in these rearranged gene segments when they could be compared to germ-line sequences. Lines expressing a particular V_H family used a variety of V_κ families, suggesting that the expressed heavy chain in a cell does not influence the choice of V_κ rearrangements. In particular, four hybridoma lines expressing V_H7183 had rearranged gene segments from four V_κ families, and the 18-81 line expressing V_H3660 (21) had rearranged gene segments from six V_κ families. A high frequency of $J_\kappa 5$ usage was observed in the 18-81 subclones compared with the distribution of J_κ gene segments in the hybridomas. The frequent use of $J_\kappa 5$ could be caused by secondary rearrangements on a single allele in which an initial *V*-to-*J* rearrangement is replaced by an upstream germ-line V_κ gene segment rearranging to a downstream germ-line J_κ gene segment (22–24). The frequency of secondary rearrangements *in vivo* is not known; however, the preferential use of $J_\kappa 1$ and $J_\kappa 2$ seen in adult splenic B cells (25, 26) suggests a low rate. Although the J_κ usage in our hybridomas is not biased toward $J_\kappa 1$ and $J_\kappa 2$, the population is small, and a larger sampling of V_κ rearrangements from fetal and neonatal B cells may demonstrate a bias to $J_\kappa 1$ and $J_\kappa 2$.

In general, the patterns of V_κ rearrangements were similar between the hybridomas and 18-81 subclones in that families from across the locus were utilized and certain gene segments were repeatedly rearranged. Fourteen of the V_κ gene segments were unique, and five were repeated. Among the repeated gene segments, four were rearranged twice: $V_\kappa V105$, $V_\kappa H9$, $V_\kappa 21E1.5$, and $V_\kappa A5$; and one was rearrranged three times: $V_\kappa 10ArsA$. $V_\kappa V105$ and $V_\kappa 10ArsA$ were seen in both the hybridomas and the 18-81 subclones. Although the patterns of V_κ rearrangements are not biased to families located in one region of the locus, the repeated use of V_κ1, V_κ4, V_κ10, V_κ21, and V_κ24 families may indicate preferential use of several families that could become apparent in a larger sampling of rearrangements.

DISCUSSION

Repeated Rearrangements of Individual Gene Segments Suggest Gene Targeting. The observation of five repeatedly rearranged gene segments suggests that the process of rearrangement is not randomly distributed over the entire V_κ repertoire. Using established statistical techniques (27) to estimate the size of the fetal V_κ gene pool from our data, a probability distribution was calculated that reached a maximum of 41 gene segments with a 95% confidence interval up

Hybridomas						18-81 subclones					
V_κ family	Cell line	Size	V_κ gene	J_κ gene	Type	V_κ family	Cell line	Size	V_κ gene	J_κ gene	Type
1	DA4-6	3.3	V105	2	VJ+	1	1H6A	2.7	V105	4	VJ+
	ID3-2	6.0	1.60	2	VJ+						
4	MD2-16	5.8	4.58	4	VJ+	4	T17B	2.2	H13	5	VJ+
	4-1	3.0	H1	2	VJ+		T17B	3.0	4.30	5	VJ−
	45-1	6.8	4.68	2	VJ+		T17B	4.6	R11	5	VJ−
							T17B	2.5	H9	4	VJ−
9	15-56-1	4.2	9.42	1	VJ+		T29B5-2	3.4	H9	1	VJ−
10	134-1	3.8	10ArsA	2	VJ+	10	1H6A	3.8	10ArsA	2	VJ+
	GB3-1	3.2	10ArsA	4	VJ+						
12	BC2-5-12	4.8	K2	5	VJ+	19	T4B20	3.4	19.34	5	VJ−
21	BM32-1-6	5.3	21E1.5	5	VJ−	23	T24B	3.2	23.32	5	VJ−
	BFL14	5.6	21E1.5	4	VJ−						
	BC2-5-12	12.0	21E1.6	2	VJ+	24	T17B	4.8	A5	5	VJ+
							16C	4.8	A5	5	VJ+
							1H6A	7.5	M167	5	VJ+
							2E5A	2.3	24.23	5	VJ−

FIG. 1. V_κ rearrangements in fetal and neonatal B-cell hybridomas and 18-81 subclones. Rearrangements are grouped according to family (12). Size indicates the length in kb of each *Hin*dIII fragment. Previously identified gene segments are as follows: *V105* (13); *H1*, *H9*, *H13*, and *R11* (14); *10ArsA* (15); *21E1.5* and *21E1.6* (16); *M167* (17); *A5* (18); and *K2* (19). The *K2* sequence matches the published sequence including 60 nucleotides of 5′ flanking region except for a thymine to adenine change in codon 16. Gene segments found in this study are designated as "family.size." Nine rearrangements are designated nonproductive (VJ−) because the *VJ* junctions either contained a stop codon or engaged the J_κ gene segment in a reading frame that would generate a stop codon in the constant gene. Productive rearrangements are noted VJ+. Independent rearrangements of identical V_κ gene segments are outlined in boxes.

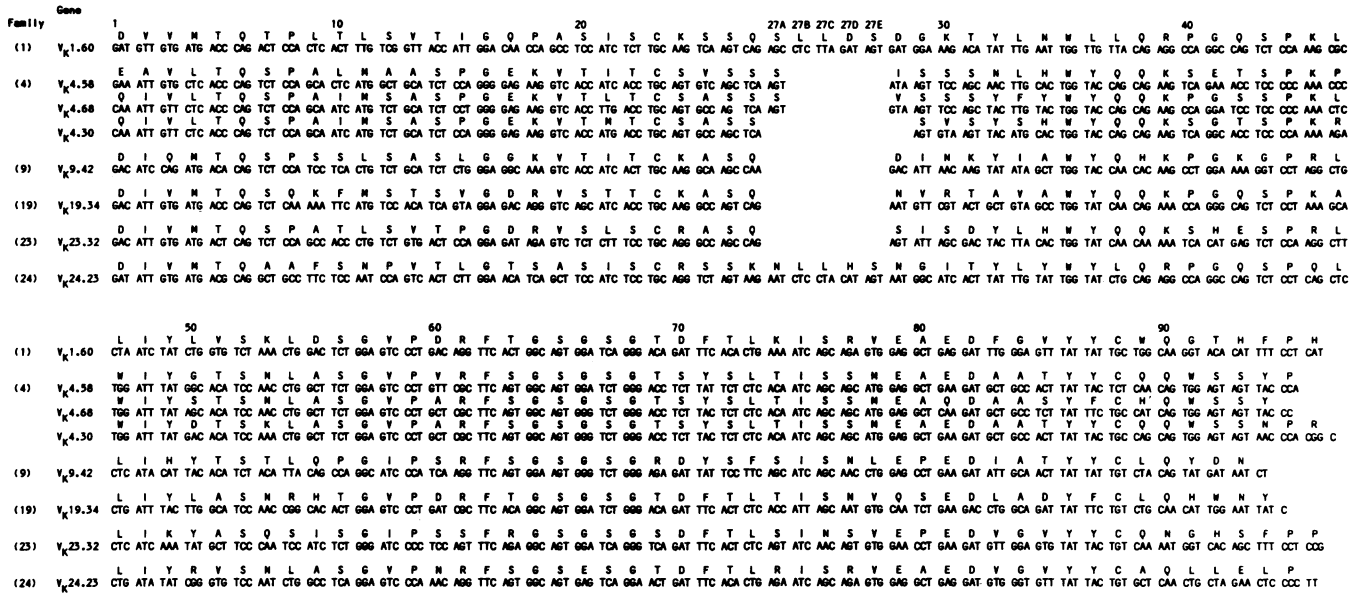

FIG. 2. Nucleotide and predicted amino acid sequences of newly identified V_κ gene segments. Gene segments are grouped according to the family shown in parentheses.

to 110 gene segments. Because previous estimates of the total germ-line V_κ repertoire range from 100 to 300 gene segments, the low pool size calculated from our data suggests that a small portion of the V_κ repertoire is rearranged more frequently than the rest. Because the 18-81 cell line rearranged V_κ gene segments *in vitro*, its repeated rearrangements were not due to antigen selection but are probably due to the rearrangement process itself. Clearly, the phenomenon of repeated rearrangements of individual gene segments that was seen on the V_H locus (3, 5, 9) is also occurring on the V_κ locus. The function or control of this process remains unknown. However, repeated rearrangements may be caused by variations in flanking DNA sequences that increase the binding affinity for recombination enzymes. Comparison of the heptamer and nonamer recombination recognition sequences in several repeated gene segments, V_H81X (3), V_HOx2 (28), $V_\kappa10ArsA$ (15), and $V_\kappa21E1.5$ (16), revealed no consistent substitutions relative to the consensus sequences. The possibility remains that several independent changes within the heptamer and nonamer sequences or changes in adjoining regions may effect comparable increases in the binding affinity of recombination enzymes and target rearrangement to individual gene segments. Alternatively, the local chromatin structure around certain gene segments may make them more accessible to recombination enzymes (29).

V_κ Rearrangements Use Families Distributed Throughout the Locus. The patterns of rearrangements were compared to a genetic map of the V_κ locus (2). The distributions are shown in Fig. 3 along with data of V_H rearrangements from comparable cell lines (see Fig. 3 legend). The V_κ rearrangements were dispersed throughout the locus (Fig. 3*D* and *E*), in contrast to the biased distributions of V_H rearrangements from both fetal and neonatal hybridomas (Fig. 3*A*) and Abelson virus-transformed cell lines that rearranged V_H gene segments *in vitro* (Fig. 3*B*). Although the exact position of the individual V_κ gene segments is not known, it is unlikely that the 19 gene segments from nine families that we observed are clustered next to the J_κ locus. Similarly, Kaushik *et al.* (31) examined V_κ expression in splenocyte colonies from neonatal C57BL/6 mice and also found use of V_κ families located across the locus. They observed a higher frequency of $V_\kappa1$ and $V_\kappa9$ expression and a lower frequency of $V_\kappa4$, $V_\kappa21$, and $V_\kappa24$ expression than reported here; the disparity may be due to dissimilar strains of mice, their use of 6- to 8-day-old mice, or different assays.

Several models may explain the broader pattern of V_κ rearrangements compared with V_H rearrangements. (*i*) Accessibility. Because V_κ gene segments rearrange later than V_H gene segments, the chromatin structure of the V_κ locus may change with time to allow recombination enzymes entry into more distal sites. Thus, a larger region of the locus may be accessible to rearrangement than on the V_H locus. (*ii*) Inversion. Approximately half of the V_κ gene segments are believed to lie in the opposite transcriptional orientation on the chromosome as the J_κ gene segments (24) and, therefore, rearrange by inversion of DNA (22). In contrast, the majority of V_H gene segments appear to lie in the same transcriptional orientation as the diversity (*D*) and J_H gene segments and rearrange by deletion (30). Inversion could engage more distal V_κ gene segments than deletion; however, there appears to be no obvious correlation between orientation and frequency of rearrangement. For example, one frequently rearranging gene, $V_\kappa21E1.5$, rearranges by deletion (16), and another, $V_\kappa10ArsA$, rearranges by inversion (24). (*iii*) Secondary rearrangement. Analogous to the heavy-chain pattern, primary rearrangements may occur to J_κ-proximal gene segments but are then replaced by secondary rearrangements of upstream V_κ to downstream J_κ gene segments. Heavy-chain gene segments are less likely to undergo secondary rearrangements because both *D*-to-J_H and V_H-to-DJ_H rearrangements occur by deletion, leaving no germ-line *D* gene segments available for secondary rearrangements. On the V_κ locus, an initial rearrangement would position the upstream region of the locus next to the J_κ gene segments, perhaps making upstream V_κ gene segments more accessible for participation in secondary rearrangements. Examples have been reported where secondary rearrangements used either different V_κ families than the initial rearrangement (23, 32), or the same V_κ family (24). Thus, secondary rearrangements may contribute to the diversity of the V_κ repertoire *in vivo*.

Evidence for the temporal expression of immunoglobulins during ontogeny is seen by the appearance of B cells specific for certain antigens at reproducible times (33). It is tempting to speculate that the programmed appearance of antibodies is due to developmentally activated gene rearrangements. Our data indicate that usage of V_κ gene segments is more heterogenous than usage of V_H gene segments during early on-

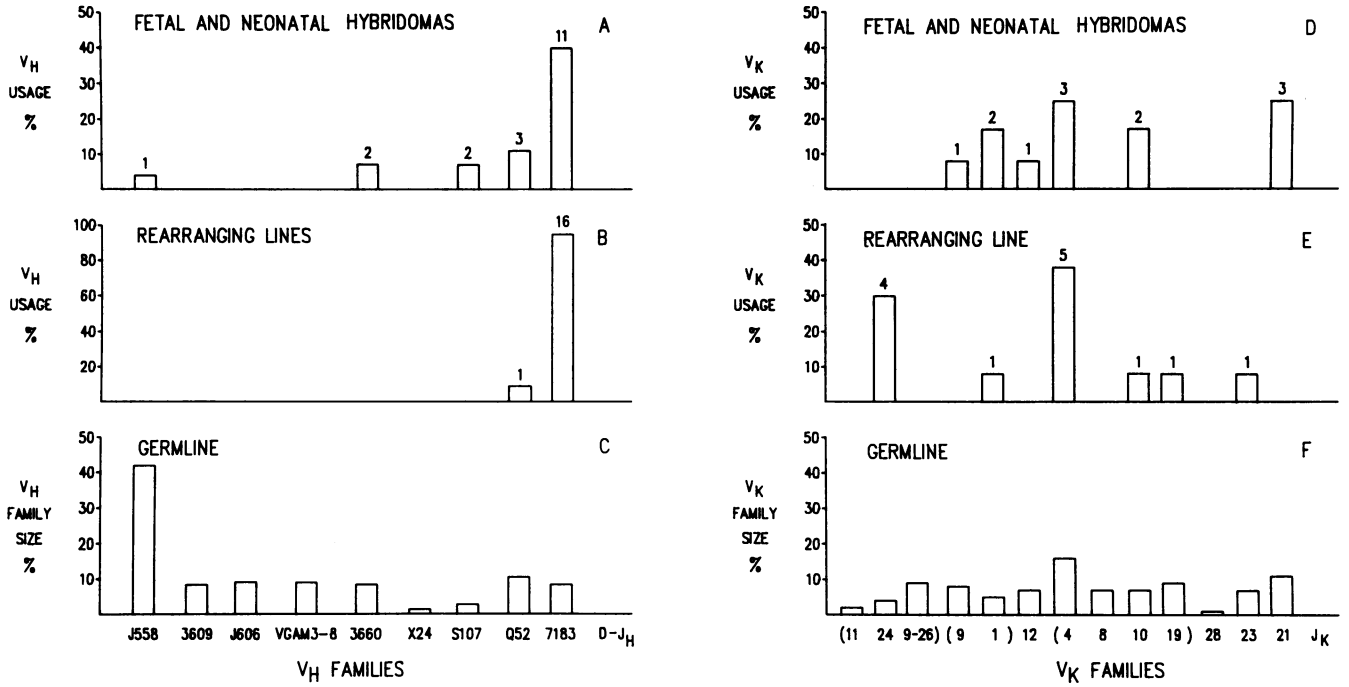

FIG. 3. Schematic diagram of distributions of germ-line and rearranged V_H and V_κ gene segments from BALB/c mice. Relative positions of the variable gene families are shown along the *y* axes. Both deletional and recombinant inbred strain analyses have been used to map these loci (2, 30); the order of families within parentheses is not known. The size of families in *C* and *F* was estimated from the number of restriction fragments hybridizing to family-specific probes on standard Southern blots (2, 30). Numbers at the top of each box in *A*, *B*, *D*, and *E* represent the number of rearrangements for that family. (*A*) V_H rearrangements in B-cell (μ^+L^+) hybridomas from fetal and day-1 neonatal liver (ref. 4 and A.M.L., unpublished results). Eight unidentified rearrangements are included in the calculation of % V_H usage. (*B*) V_H rearrangements that occurred during culture of Abelson virus-transformed pre-B cell lines (3). (*D*) Hybridoma data from Fig. 1. (*E*) 18–81 subclone data from Fig. 1.

togeny, which should produce a diverse repertoire of B cells. It remains to be seen whether the temporal expression of certain antibodies is due to delayed rearrangement of their individual V_κ and V_H gene segments.

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