Brief Definitive Report

MURINE V_κ GENE EXPRESSION DOES NOT FOLLOW THE V_H PARADIGM

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The germline V gene segments from which the functional H and L chain genes are constructed have been classified into families based upon the amino acid sequence of mAbs (1, 2) and by DNA sequence homology (3, 4). Thus, the estimated 100–1,000 H chain V gene segments (V_H) have been classified into 11 families (3, 5–7), while the 100–300 V gene segments (8) of the κ L chain (V_κ) have been divided into 29 subgroups or families (2, 9). Analysis by a variety of independent methods (10–15) indicates that, in general, the frequencies at which V_H families are used in adult mice is proportional to each V_H family's size. However, this does not seem to be the case in the murine fetal liver and neonatal spleen where biased usage of 3′ V_H gene families, those nearest the D and J loci, is found (16, 17). It has been suggested that these differences in V_H expression reflect developmentally controlled changes in the accessibility of the V_H locus to a recombination mechanism that exhibits a 3′ → 5′ tracking behavior (15). In contrast, little is known about V_κ usage. Since 95% of all murine antibodies bear the κ L chain (18), the role of V_κ exons in the generation of antibody diversity almost equals that of the V_H gene segments. The mode of V_κ expression in adult and neonatal mice is also unknown. For these reasons, we have determined the frequencies at which 10 V_κ families are expressed in adult and neonatal C57BL/6 mice.

Materials and Methods

Mice. Neonatal (6–8 d old) and adult (14–24 wk old) C57BL/6 mice were obtained from The Jackson Laboratories (Bar Harbor, ME) and maintained at the University of Texas Medical Branch. Thymocyte donors were sex-matched, young (5–8 wk) C57BL/6 mice.

DNA Probes. 10 V_κ gene probes, each a prototype of the V_κ1, -2, -4, -8, -10, -19, -21, -22, or -24 families as well as a C_μ-specific probe have been described (12, 19). A C_κ-specific probe, a 3.1-kb Bam HI, Hind III fragment containing the genomic C_κ sequence was derived from the plasmid pC_κ, the generous gift of Dr. P. Tucker (University Texas Health Science Center, Dallas, TX).

B Lymphocyte Cloning. Colonies of B cells, representing the progeny of single mitogen-reactive lymphocytes, were grown in vitro on filter paper discs as described (20). Briefly, splenocytes were plated at low densities (10^5 cells) onto filter paper discs and cultured in the presence of 20 μg/ml LPS and 3 × 10^7 isologous thymocyte feeder cells. After 5 d of culture, discs were fixed in neutral buffered formalin, washed in 0.1 × PBS and air dried.

In Situ Hybridization. Briefly, discs were rinsed in chloroform/isoamyl alcohol (24:1), washed three times in 0.1 × PBS/0.1% SDS, and prehybridized overnight (50% formamide, 5 × SSC, 5 × Denhardt's solution, 50 mM phosphate buffer (pH 6.5), 1% glycine, 0.5% SDS and 50 μg/ml salmon sperm DNA). Subsequently, a 48-h hybridization was performed with 1–2 ×

10^5 cpm/ml of ^{32}P-oligolabeled V_κ-specific DNA probes. After stringent washing, discs were autoradiographed for 7 d on Kodak films as described (12). After stripping bound counts (12), the same discs were again hybridized with C_μ- or C_κ-specific probes to reveal all B cell colonies. The frequency of V_κ families was determined by scoring the number of clones hybridizing with a particular V_κ probe divided by total number of C_μ^+ or C_κ^+ clones.

Results

Hybridizations using either the C_μ or C_κ probes show no significant differences in the expression of the $V_\kappa 1$ gene family (Table I), indicating that either probe serves equally well to detect B cell colonies. This result is expected since LPS-driven colony formation predominantly expands IgM-bearing (C_μ^+) B cells (21) and since the κ isotype is expressed on ≥95% of all murine B lymphocytes (18). Thus, we shall describe colonies hybridizing with either the C_κ- or C_μ-specific probe as "C^+".

Nonstoichiometric V_κ Gene Expression in Adult Mice. Frequencies at which 10 V_κ gene families are expressed among B cell colonies derived from C57BL/6 mice are presented in Table II. Large numbers (28,106) of C^+ colonies were screened in four independent experiments to ensure detection of infrequently expressed V_κ families and to establish the degree of intrastrain variability. The $V_\kappa 1$ gene family is most prevalent, expressed in more than one-quarter of all B cell colonies. In contrast, $V_\kappa 24$ gene segments are expressed in only 0.3% of C^+ colonies, a frequency almost 100-fold below that for $V_\kappa 1$ (Table II). Surprisingly, unlike V_H expression, utilization of V_κ gene families does not approximate stoichiometric use. Of the V_κ families examined in this census, the $V_\kappa 8$, -9, -19, and -21 families are the largest as determined by their genomic complexity (12, 11, 10, and 10 members respectively; Table II). However, none of these families are expressed at frequencies >10% in adult mice (Table II). Indeed, the most and least frequently expressed V_κ families, $V_\kappa 1$ and $V_\kappa 24$, have similar complexities, 3 and 2, respectively. Finally, the 10 V_κ gene family probes used in these experiments accounted for about 60% of all C^+ LPS-induced B colonies derived from adult mice.

V_κ Gene Expression in Neonates Is not Biased for 3′ Families. Analysis in three experiments of 18,462 colonies of B cells taken from neonatal mice (Fig. 1) revealed several important differences. First, significant increases in the frequencies of $V_\kappa 1$ and $V_\kappa 9$ (~2-fold and 5-fold, respectively [$p \leq 0.05$]) were seen along with less dramatic increases in the expression of $V_\kappa 8$ and $V_\kappa 4$ exons (Table II). Second, the $V_\kappa 19$ and $V_\kappa 22$ gene families were observed at lower frequencies (~5-fold and 40-fold, respectively [$p \leq 0.01$]) in neonatal vs. adult mice. Interestingly, the 10 V_κ probes used accounted for 89% of all C^+ colonies screened. However, our most striking observation was the failure to detect expression of $V_\kappa 21$ exons (0/4,490; Table II) among colonies of B cells derived from neonates. The $V_\kappa 21$ gene family has been mapped most proximal to the J_κ locus (11) and might have been expected to enjoy the biased expression of the analogous 3′ V_H gene family, V_H 7183 (16, 17).

Discussion

Among murine antibodies, the κ L chain is dominant (18); thus V_κ exons are virtually equal in importance to the V_H exons in creating antibody diversity. The murine *Igκ* locus is located on chromosome 6 and is thought to contain some 100–300 V_κ exons that are organized into discrete families of reiterated homologous sequences (9). We have used 10 gene probes specific for the $V_\kappa 1$, -2, -4, -8, -9, -19, -21, -22,

TABLE I

Comparison of $V_\kappa 1$ Expression Among C_κ^+ or C_μ^+ Colonies

Nos. $V_\kappa 1^+$	Nos. C_κ^+	Nos. C_μ^+	Average frequency*
211 (n = 7)‡	648	–	33 ± 8%
303 (n = 10)	–	942	32 ± 9%

* Represents the mean (± SD) frequency of $V_\kappa 1$ expression.
‡ *n*, Number of discs screened.

TABLE II

V_κ Gene Family Use Among LPS-activated Splenocyte Colonies from Adult and Neonatal C57BL/6 Mice

Gene order:* centromere Hd-/ $V_\kappa 2$;	$V_\kappa 22$/ -($V_\kappa 11$;	$V_\kappa 24$; $V_\kappa 9$-26)- ($V_\kappa 1$;	$V_\kappa 9$) - ($V_\kappa 4$;	$V_\kappa 8$;	$V_\kappa 10$; $V_\kappa 12$-13;	$V_\kappa 19$)-($V_\kappa 28$; Rn7s-6)-$V_\kappa 23$-($V_\kappa 21$ -J_κ-C_κ)	
Genomic complexity:‡	5	7	2	3	11	8	12	5	10	10
	$V_\kappa 2$	$V_\kappa 22$	$V_\kappa 24$	$V_\kappa 1$	$V_\kappa 9$	$V_\kappa 4$	$V_\kappa 8$	$V_\kappa 10$	$V_\kappa 19$	$V_\kappa 21$
V_κ expression (V_κ/C_μ or C_κ)	35	47	10	924	105	93	371	14	307	63
Adult:	2,126	1,090	3,780	3,582	2,064	2,848	4,125	1,035	5,040	2,429
	1.7%	4.3%	0.3%	25.8%	5.1%	3.3%	9.0%	1.4%	6.1%	2.6%
Neonatal:	44	5	0	616	241	59	162	3	42	0
	928	2,929	3,086	1,538	1,049	1,093	1,174	918	3,512	4,490
	4.7%	0.2%	–	40.1%	23.0%	5.4%	13.8%	0.3%	1.2%	–

Neonatal mice 6–8 d old; adult mice 14–24 wk old.
* From reference 9. Gene order within parentheses is not known. The $V_\kappa 2$ and $V_\kappa 22$ families are unmapped. Hd, Hypodactyly. Rn7s-6, 7s ribonucleoprotein.
‡ From reference 19. Complexities determined by RFLP analyses of genomic DNA cut with Bam HI, HinD III, or both.

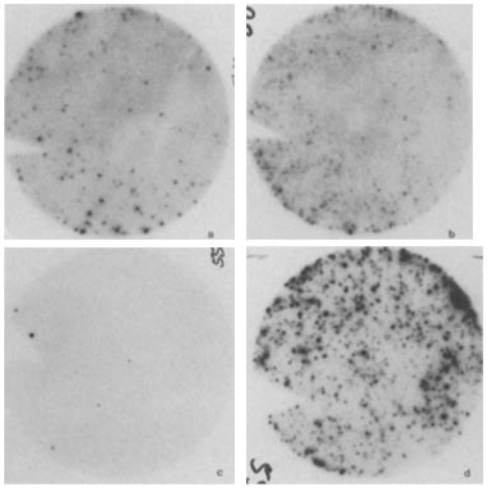

FIGURE 1. Sequential hybridizations of the $V_\kappa 1$ or $V_\kappa 22$ and C_μ probes to LPS-induced B cell colonies from neonatal C57BL/6. Note that the frequency of $V_\kappa 1^+$ (*a* and *b*) greatly exceeds $V_\kappa 22^+$ colonies (*c* and *d*). Disc A was probed with $V_\kappa 1$ (*a*) followed by C_μ (*b*) after stripping. Similarly, disc B (*c* and *d*) was hybridized to $V_\kappa 22$- and C_μ-specific probes.

or $V_\kappa 24$ gene families to investigate V_κ expression in C57BL/6 mice. By RFLP analysis of genomic DNA (19), our probes account for 73 of the 100–300 V_κ exons. Thus, while not exhaustive, this study addresses a meaningful fraction of the V_κ gene segments.

Our census of some 4.7×10^4 B cell colonies derived from neonatal and adult C57BL/6 mice has identified age-specific patterns of V_κ expression (Table II). In adult C57BL/6 mice the 10 V_κ gene families studied accounted for about 60% of all C^+ colonies screened, a value consistant with estimates of the number of V_κ exons. Most of the 10 V_κ gene families were expressed at levels <10%. The exception, $V_\kappa 1$, was transcribed in almost 26% of colonies ($V_\kappa 1 > V_\kappa 8 > V_\kappa 19 \geq V_\kappa 9 \geq V_\kappa 22 \geq V_\kappa 4 \geq V_\kappa 21 \geq V_\kappa 2 \geq V_\kappa 10 > V_\kappa 24$). This observation is in agreement with the higher than expected frequency of $V_\kappa 1$ expression among myeloma libraries (21) and within certain responses to self antigens (22). In contrast, the same 10 V_κ gene families accounted for almost 90% of B cell colonies derived from 6–8-d-old C57BL/6 mice. Three V_κ gene families, $V_\kappa 1$, $V_\kappa 9$, and $V_\kappa 8$, alone made up the majority (77%) of early κ L chain expression ($V_\kappa 1 > V_\kappa 9 > V_\kappa 8 > V_\kappa 4 \sim V_\kappa 2 > V_\kappa 19 > V_\kappa 10 \geq V_\kappa 22 > V_\kappa 24 \sim V_\kappa 21$). This circumscription of V_κ usage and the contemporary bias for the expression of 3′ V_H gene segments (16, 17) is likely to be an important element in the limited antibody diversity found in neonatal mice (23).

Our results also illustrate that V_κ gene family expression differs from that of V_H expression in at least two important respects. First, in adult C57BL/6 mice, V_κ family expression is not correlated to family size. This is in contrast to V_H expression in adult mice where V_H family usage and genomic complexity correlate well (11, 12). However, we stress that measures of genomic complexity are not an enumeration of V_κ segments and may not precisely reflect the number of functional exons within a V_κ family (8). In addition, we can not formally exclude biased expansion of certain B cells (e.g., $V_\kappa 1^+$) by LPS or inappropriate hybridization by some number of our probes. However, LPS has not been found to bias V_H expression (10, 12–14) and with Southern blots no cross (interfamily) hybridization was observed between the 10 V_κ probes used (data not shown). For these reasons, we are convinced that

V_κ expression in adult mice is not stoichiometric. Second, V_κ usage in neonatal C57BL/6 mice does not reflect a positional bias for the expression of J_κ-proximal exons. Although the organization of the *Igκ* locus has not yet been precisely defined, recombinational analyses by D'Hoostelaere et al. (9) have generated the genetic map depicted in Table II. The $V_\kappa 1$, -9, and -8 gene families, which alone account for almost 80% of the early κ L chains, map near the center of the *Igκ*-V locus. Indeed, the V_κ family mapped most proximal to the J_κ locus, $V_\kappa 21$, is rarely, if at all, expressed (<1/4,490) in the neonate.

These contrasts imply that the mechanisms for V_κ gene rearrangement and expression may differ from those controlling the V_H locus. For example, unlike the *Igh* locus, analyses of plasmacytomas suggest that many V_κ exons lie in a transcriptional orientation opposite that of the J_κ locus (24). Although the import of such findings remains unclear, Alt and his colleagues have proposed a model for Ig rearrangement and expression (15) based upon a universal recombinase that tracks across "accessible" portions of the Ig loci in a 3'→5' direction. As the two V_κ families most frequently expressed in neonates, $V_\kappa 1$ and $V_\kappa 9$, map adjacent to one another, positional bias may influence early V_κ expression. However, the process of developmentally regulated V_κ expression is undoubtedly more complex than can be explained by the linear tracking models currently proposed.

Summary

V_κ gene family expression among LPS-reactive murine B lymphocytes, unlike that of V_H gene families, is not proportional to genomic complexity, i.e., nonstoichiometric. Furthermore, no positional bias for the overexpression of J-proximal V_κ genes ($V_\kappa 21$) is observed among neonatal B lymphocytes. Yet, the $V_\kappa 1$ and $V_\kappa 9$ families located in the center of V_κ locus are preferentially used by neonatal B splenocytes. Thus, the mechanisms of V_κ gene rearrangement and expression appear to differ significantly from those controlling the V_H locus.

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