

**MURINE V<sub>κ</sub> GENE EXPRESSION DOES NOT FOLLOW  
THE V<sub>H</sub> 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<sub>H</sub>) have been classified into 11 families (3, 5–7), while the 100–300 V gene segments (8) of the κ L chain (V<sub>κ</sub>) 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<sub>H</sub> families are used in adult mice is proportional to each V<sub>H</sub> 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<sub>H</sub> gene families, those nearest the D and J loci, is found (16, 17). It has been suggested that these differences in V<sub>H</sub> expression reflect developmentally controlled changes in the accessibility of the V<sub>H</sub> locus to a recombination mechanism that exhibits a 3' → 5' tracking behavior (15). In contrast, little is known about V<sub>κ</sub> usage. Since 95% of all murine antibodies bear the κ L chain (18), the role of V<sub>κ</sub> exons in the generation of antibody diversity almost equals that of the V<sub>H</sub> gene segments. The mode of V<sub>κ</sub> expression in adult and neonatal mice is also unknown. For these reasons, we have determined the frequencies at which 10 V<sub>κ</sub> 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<sub>κ</sub> gene probes, each a prototype of the V<sub>κ</sub>1, -2, -4, -8, -10, -19, -21, -22, or -24 families as well as a C<sub>κ</sub>-specific probe have been described (12, 19). A C<sub>κ</sub>-specific probe, a 3.1-kb Bam HI, Hind III fragment containing the genomic C<sub>κ</sub> sequence was derived from the plasmid pC<sub>κ</sub>, 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<sup>5</sup> cells) onto filter paper discs and cultured in the presence of 20 μg/ml LPS and 3 × 10<sup>7</sup> 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  $^{32}\text{P}$ -oligolabeled  $V_{\kappa}$ -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  $C_{\mu}$ - or  $C_{\kappa}$ -specific probes to reveal all B cell colonies. The frequency of  $V_{\kappa}$  families was determined by scoring the number of clones hybridizing with a particular  $V_{\kappa}$  probe divided by total number of  $C_{\mu}^+$  or  $C_{\kappa}^+$  clones.

## Results

Hybridizations using either the  $C_{\mu}$  or  $C_{\kappa}$  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 ( $C_{\mu}^+$ ) B cells (21) and since the  $\kappa$  isotype is expressed on  $\geq 95\%$  of all murine B lymphocytes (18). Thus, we shall describe colonies hybridizing with either the  $C_{\kappa}$ - or  $C_{\mu}$ -specific probe as " $C^+$ ".

*Nonstoichiometric  $V_{\kappa}$  Gene Expression in Adult Mice.* Frequencies at which 10  $V_{\kappa}$  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  $V_{\kappa}$  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  $V_{\kappa}$  gene families does not approximate stoichiometric use. Of the  $V_{\kappa}$  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  $V_{\kappa}$  families,  $V_{\kappa}1$  and  $V_{\kappa}24$ , have similar complexities, 3 and 2, respectively. Finally, the 10  $V_{\kappa}$  gene family probes used in these experiments accounted for about 60% of all  $C^+$  LPS-induced B colonies derived from adult mice.

*$V_{\kappa}$  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  $V_{\kappa}9$  ( $\sim 2$ -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 ( $\sim 5$ -fold and 40-fold, respectively [ $p \leq 0.01$ ]) in neonatal vs. adult mice. Interestingly, the 10  $V_{\kappa}$  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  $J_{\kappa}$  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  $\kappa$  L chain is dominant (18); thus  $V_{\kappa}$  exons are virtually equal in importance to the  $V_H$  exons in creating antibody diversity. The murine  $Ig\kappa$  locus is located on chromosome 6 and is thought to contain some 100–300  $V_{\kappa}$  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  $V_{k1}$  Expression Among  $C_{k^+}$  or  $C_{\mu^+}$  Colonies

Nos. $V_{k1}^+$	Nos. $C_{k^+}$	Nos. $C_{\mu^+}$	Average frequency*
211 (n = 7)†	648	—	33 ± 8%
303 (n = 10)	—	942	32 ± 9%

\* Represents the mean (± SD) frequency of  $V_{k1}$  expression.

† n, Number of discs screened.

TABLE II  
 $V_k$  Gene Family Use Among LPS-activated Splenocyte Colonies from Adult and Neonatal C57BL/6 Mice

Gene order:*	Hd/ $V_{k2}$	$V_{k22}/(V_{k11}; V_{k24}; V_{k9-26)$	$(V_{k1}; V_{k9}) - (V_{k4}; V_{k8}; V_{k10}; V_{k12-13}; V_{k19}) - (V_{k28}; Rn7s-6) - V_{k23} - (V_{k21} - J_{k-C_{k1}})$							
Genomic complexity:†	5	7	2	3	11	8	12	5	10	10
$V_k$ expression ( $V_k/C_{\mu}$ or $C_k$ )	$V_{k2}$	$V_{k22}$	$V_{k24}$	$V_{k1}$	$V_{k9}$	$V_{k4}$	$V_{k8}$	$V_{k10}$	$V_{k19}$	$V_{k21}$
Adult:	2,126 1.7%	1,090 4.3%	3,780 0.3%	924 25.8%	105 5.1%	93 3.3%	371 9.0%	14 1.4%	307 6.1%	63 2.6%
Neonatal:	44 4.7%	5 0.2%	0 —	616 40.1%	241 23.0%	59 5.4%	162 13.8%	3 0.3%	42 1.2%	0 —
	928 4.7%	2,929 0.2%	3,086 —	1,538 40.1%	1,049 23.0%	1,093 5.4%	1,174 13.8%	918 0.3%	3,512 1.2%	4,490 —

Neonatal mice 6-8 d old; adult mice 14-24 wk old.

\* From reference 9. Gene order within parentheses is not known. The  $V_{k2}$  and  $V_{k22}$  families are unmapped. Hd, Hypodactyl. Rn7s-6, 7s ribonucleoprotein.

† From reference 19. Complexities determined by RFLP analyses of genomic DNA cut with Bam HI, Hind III, or both.

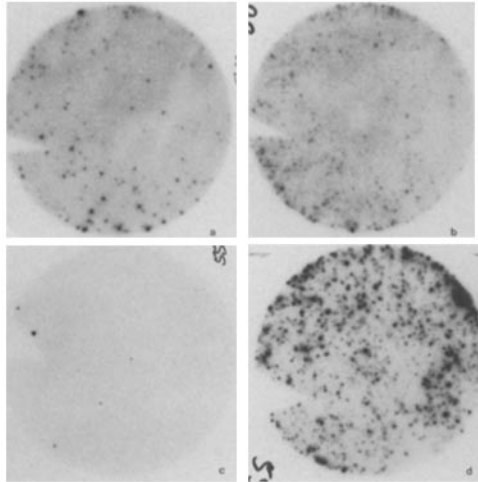


FIGURE 1. Sequential hybridizations of the  $V_{\kappa}1$  or  $V_{\kappa}22$  and  $C_{\mu}$  probes to LPS-induced B cell colonies from neonatal C57BL/6. Note that the frequency of  $V_{\kappa}1^{+}$  (a and b) greatly exceeds  $V_{\kappa}22^{+}$  colonies (c and d). Disc A was probed with  $V_{\kappa}1$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

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

Our census of some  $4.7 \times 10^4$  B cell colonies derived from neonatal and adult C57BL/6 mice has identified age-specific patterns of  $V_{\kappa}$  expression (Table II). In adult C57BL/6 mice the 10  $V_{\kappa}$  gene families studied accounted for about 60% of all  $C^{+}$  colonies screened, a value consistent with estimates of the number of  $V_{\kappa}$  exons. Most of the 10  $V_{\kappa}$  gene families were expressed at levels  $<10\%$ . The exception,  $V_{\kappa}1$ , was transcribed in almost 26% of colonies ( $V_{\kappa}1 > V_{\kappa}8 > V_{\kappa}19 \gg V_{\kappa}9 \gg V_{\kappa}22 \gg V_{\kappa}4 \gg V_{\kappa}21 \gg V_{\kappa}2 \gg V_{\kappa}10 > V_{\kappa}24$ ). 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  $V_{\kappa}$  gene families accounted for almost 90% of B cell colonies derived from 6–8-d-old C57BL/6 mice. Three  $V_{\kappa}$  gene families,  $V_{\kappa}1$ ,  $V_{\kappa}9$ , and  $V_{\kappa}8$ , alone made up the majority (77%) of early  $\kappa$  L chain expression ( $V_{\kappa}1 > V_{\kappa}9 > V_{\kappa}8 > V_{\kappa}4 \sim V_{\kappa}2 > V_{\kappa}19 > V_{\kappa}10 \gg V_{\kappa}22 > V_{\kappa}24 \sim V_{\kappa}21$ ). This circumscription of  $V_{\kappa}$  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  $V_{\kappa}$  gene family expression differs from that of  $V_H$  expression in at least two important respects. First, in adult C57BL/6 mice,  $V_{\kappa}$  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  $V_{\kappa}$  segments and may not precisely reflect the number of functional exons within a  $V_{\kappa}$  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  $V_{\kappa}$  probes used (data not shown). For these reasons, we are convinced that

$V_{\kappa}$  expression in adult mice is not stoichiometric. Second,  $V_{\kappa}$  usage in neonatal C57BL/6 mice does not reflect a positional bias for the expression of  $J_{\kappa}$ -proximal exons. Although the organization of the *Ig $\kappa$*  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  $\kappa$  L chains, map near the center of the *Ig $\kappa$ -V* locus. Indeed, the  $V_{\kappa}$  family mapped most proximal to the  $J_{\kappa}$  locus,  $V_{\kappa}21$ , is rarely, if at all, expressed (<1/4,490) in the neonate.

These contrasts imply that the mechanisms for  $V_{\kappa}$  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  $V_{\kappa}$  exons lie in a transcriptional orientation opposite that of the  $J_{\kappa}$  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  $V_{\kappa}$  families most frequently expressed in neonates,  $V_{\kappa}1$  and  $V_{\kappa}9$ , map adjacent to one another, positional bias may influence early  $V_{\kappa}$  expression. However, the process of developmentally regulated  $V_{\kappa}$  expression is undoubtedly more complex than can be explained by the linear tracking models currently proposed.

### Summary

$V_{\kappa}$  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  $V_{\kappa}$  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  $V_{\kappa}$  locus are preferentially used by neonatal B splenocytes. Thus, the mechanisms of  $V_{\kappa}$  gene rearrangement and expression appear to differ significantly from those controlling the  $V_H$  locus.

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