MURINE $V_k$ 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 ($V_K$) of the $\kappa$ L chain ($V_K$) 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 $3' \rightarrow 5'$ tracking behavior (15). In contrast, little is known about $V_K$ usage. Since 95% of all murine antibodies bear the $\kappa$ L chain (18), the role of $V_K$ exons in the generation of antibody diversity almost equals that of the $V_H$ gene segments. The mode of $V_K$ expression in adult and neonatal mice is also unknown. For these reasons, we have determined the frequencies at which 10 $V_K$ 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 $V_K$ gene probes, each a prototype of the $V_K$1, -2, -4, -8, -10, -19, -21, or -24 families as well as a $C_k$-specific probe have been described (12, 19). A $C_k$-specific probe, a 3.1-kb Bam HI, Hind III fragment containing the genomic $C_k$ sequence was derived from the plasmid pCk, 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 ($10^3$ cells) onto filter paper discs and cultured in the presence of 20 $\mu$g/ml LPS and 3 x 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 $\mu$g/ml salmon sperm DNA). Subsequently, a 48-h hybridization was performed with 1–2 ×

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10^3 cpm/ml of 32P-oligolabeled V\(_k\)-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\(_k\)-specific probes to reveal all B cell colonies. The frequency of V\(_k\) families was determined by scoring the number of clones hybridizing with a particular V\(_k\) probe divided by total number of C\(_\mu\) or C\(_k\) clones.

**Results**

Hybridizations using either the C\(_\mu\) or C\(_k\) probes show no significant differences in the expression of the V\(_k\)-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 \(k\) isotype is expressed on \(\geq 95\%\) of all murine B lymphocytes (18). Thus, we shall describe colonies hybridizing with either the C\(_k\) or C\(_\mu\)-specific probe as “C+”.

**Nonstoichiometric V\(_k\) Gene Expression in Adult Mice.** Frequencies at which 10 V\(_k\) 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\(_k\) families and to establish the degree of intrastrain variability. The V\(_k\)-1 gene family is most prevalent, expressed in more than one-quarter of all B cell colonies. In contrast, V\(_k\)-24 gene segments are expressed in only 0.3% of C+ colonies, a frequency almost 100-fold below that for V\(_k\)-1 (Table II). Surprisingly, unlike V\(_\mu\) expression, utilization of V\(_k\) gene families does not approximate stoichiometric use. Of the V\(_k\) families examined in this census, the V\(_k\)-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\(_k\) families, V\(_k\)-1 and V\(_k\)-24, have similar complexities, 3 and 2, respectively. Finally, the 10 V\(_k\) gene family probes used in these experiments accounted for about 60% of all C+ LPS-induced B colonies derived from adult mice.

**Discussion**

Among murine antibodies, the \(k\) L chain is dominant (18); thus V\(_k\) exons are virtually equal in importance to the V\(_\mu\) exons in creating antibody diversity. The murine IgK locus is located on chromosome 6 and is thought to contain some 100-300 V\(_k\) exons that are organized into discrete families of reiterated homologous sequences (9). We have used 10 gene probes specific for the V\(_k\)-1, -2, -4, -8, -9, -19, -21, -22,
Table I

Comparison of V\(_4\)1 Expression Among C\(_{A}^{+}\) or C\(_{P}^{+}\) Colonies

| Nos. V\(_4\)1\(^{+}\) | Nos. C\(_{A}^{+}\) | Nos. C\(_{P}^{+}\) | Average frequency\(^{+}\) |
|---------------------|-----------------|-----------------|------------------------|
| 211                 | 648             | -               | 33 ± 8%                |
|(n = 7)\(^{1}\)      |                 |                 |                        |
| 303                 | -               | 942             | 32 ± 9%                |
|(n = 10)             |                 |                 |                        |

\(^{+}\) Represents the mean (± SD) frequency of V\(_4\)1 expression.

\(^{1}\) n, Number of discs screened.

Table II

V\(_4\) Gene Family Use Among LPS-activated Splenocyte Colonies from Adult and Neonatal C57BL/6 Mice

| Gene order: \(^{+}\) | centromere | H\(_d\)\/- V\(_4\)2; | V\(_4\)2\(-\)(V\(_4\)11; V\(_4\)12; V\(_4\)9-26)- (V\(_4\)4; V\(_4\)9) | \(-\)(V\(_4\)4; V\(_4\)9; V\(_4\)8; V\(_4\)10; V\(_4\)12-13; V\(_4\)19)-(V\(_4\)28; Rn7s-6)-V\(_4\)23-(V\(_4\)21; J\(_{e}\)-C\(_{A}\)) |
|---------------------|------------|-----------------|----------------------|----------------------|
| Genomic complexity: \(^{1}\) | 5 | 7 | 2 | 11 | 8 | 12 | 5 | 10 | 10 |
| V\(_4\)2 | V\(_4\)22 | V\(_4\)24 | V\(_4\)4 | V\(_4\)9 | V\(_4\)4 | V\(_4\)9 | V\(_4\)8 | V\(_4\)10 | V\(_4\)19 | V\(_4\)21 |
| 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\(_4\)2 and V\(_4\)22 families are unmapped. H\(_d\), Hypodactyly. Rn7s-6, 7s ribonucleoprotein.

\(^{1}\) From reference 19. Complexities determined by RFLP analyses of genomic DNA cut with Bam H\(_I\), HinD III, or both.
Figure 1. Sequential hybridizations of the VK1 or VK22 and Cκ probes to LPS-induced B cell colonies from neonatal C57BL/6. Note that the frequency of VK1' (a and b) greatly exceeds VK22' colonies (c and d). Disc A was probed with VK1 (a) followed by Cκ (b) after stripping. Similarly, disc B (c and d) was hybridized to VK22- and Cκ-specific probes.

or VK24 gene families to investigate Vκ expression in C57BL/6 mice. By RFLP analysis of genomic DNA (19), our probes account for 73 of the 100–300 Vκ exons. Thus, while not exhaustive, this study addresses a meaningful fraction of the Vκ gene segments.

Our census of some 4.7 × 10⁴ B cell colonies derived from neonatal and adult C57BL/6 mice has identified age-specific patterns of Vκ expression (Table II). In adult C57BL/6 mice the 10 Vκ gene families studied accounted for about 60% of all C⁺ colonies screened, a value consistent with estimates of the number of Vκ exons. Most of the 10 Vκ gene families were expressed at levels <10%. The exception, Vκ1, was transcribed in almost 26% of colonies (Vκ1 > Vκ8 > Vκ19 > Vκ9 > Vκ22 > Vκ4 > Vκ21 > Vκ2 > Vκ10 > Vκ24). This observation is in agreement with the higher than expected frequency of Vκ1 expression among myeloma libraries (21) and within certain responses to self antigens (22). In contrast, the same 10 Vκ gene families accounted for almost 90% of B cell colonies derived from 6–8-d-old C57BL/6 mice. Three Vκ gene families, Vκ1, Vκ9, and Vκ8, alone made up the majority (77%) of early κ L chain expression (Vκ1 > Vκ9 > Vκ8 > Vκ4 > Vκ2 > Vκ19 > Vκ10 > Vκ22 > Vκ24). This circumscription of Vκ usage and the contemporary bias for the expression of 3' Vκ 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 Vκ gene family expression differs from that of Vκ expression in at least two important respects. First, in adult C57BL/6 mice, Vκ family expression is not correlated to family size. This is in contrast to Vκ expression in adult mice where Vκ family usage and genomic complexity correlate well (11, 12). However, we stress that measures of genomic complexity are not an enumeration of Vκ segments and may not precisely reflect the number of functional exons within a Vκ family (8). In addition, we can not formally exclude biased expansion of certain B cells (e.g., Vκ1') by LPS or inappropriate hybridization by some number of our probes. However, LPS has not been found to bias Vκ expression (10, 12–14) and with Southern blots no cross (interfamily) hybridization was observed between the 10 Vκ probes used (data not shown). For these reasons, we are convinced that
\( V_k \) expression in adult mice is not stoichiometric. Second, \( V_k \) usage in neonatal C57BL/6 mice does not reflect a positional bias for the expression of \( J_k \)-proximal exons. Although the organization of the \( Igk \) 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 \( V_k1, -9, \) and \( -8 \) gene families, which alone account for almost 80% of the early \( \kappa \) L chains, map near the center of the \( Igk-V \) locus. Indeed, the \( V_k \) family mapped most proximal to the \( J_k \) locus, \( V_k21 \), is rarely, if at all, expressed (<1/4,490) in the neonate.

These contrasts imply that the mechanisms for \( V_k \) gene rearrangement and expression may differ from those controlling the \( V_h \) locus. For example, unlike the \( Igk \) locus, analyses of plasmacytomas suggest that many \( V_k \) exons lie in a transcriptional orientation opposite that of the \( J_k \) 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 \( V_k \) families most frequently expressed in neonates, \( V_k1 \) and \( V_k9 \), map adjacent to one another, positional bias may influence early \( V_k \) expression. However, the process of developmentally regulated \( V_k \) expression is undoubtedly more complex than can be explained by the linear tracking models currently proposed.

**Summary**

\( V_k \) 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 \( V_k \) genes (\( V_k21 \)) is observed among neonatal B lymphocytes. Yet, the \( V_k1 \) and \( V_k9 \) families located in the center of \( V_k \) locus are preferentially used by neonatal B splenocytes. Thus, the mechanisms of \( V_k \) gene rearrangement and expression appear to differ significantly from those controlling the \( V_h \) locus.

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