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Stochastic pairing of heavy-chain and κ light-chain variable gene families occurs in polyclonally activated B cells

(antibody repertoire/immunoglobulin gene expression)

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ABSTRACT **Frequencies of 25 immunoglobulin heavy-chain and κ light-chain variable (V_H + V_κ) gene-family pairings expressed in splenic B-cell populations were determined by hybridization of V_H- and V_κ-family-specific DNA probes to mitogen-induced B-cell colonies from C57BL/6 mice or hybridomas derived from BALB/c and NZB mice. Both analyses support the conclusion that V_H and V_κ gene families pair without bias; as would be expected for random association, the frequencies of specific V_H + V_κ pairs may be estimated by the product of the independent V_H and V_κ frequencies. Based upon the frequencies at which 9 V_H and 12 V_κ gene families are expressed, we calculated the expected usage for ≈100 V_H + V_κ family pairings in neonatal and adult C57BL/6 mice. Variability in the expression of such V_H + V_κ pairings is considerable; pairs representing >10% to <0.01% of the splenic B-cell population occur. This variability is most pronounced in the neonate, where 6 V_H + V_κ family pairs account for nearly 40% of all mitogen-reactive B cells. As the neonate matures, the distribution of frequencies for V_H + V_κ pairings becomes more nearly uniform. This process may underlie the patterned acquisition of humoral immune responsiveness.**

We and others have investigated the frequencies at which families of immunoglobulin heavy-chain and κ light-chain variable (V_H and V_κ) genes are expressed in mice during development (1–7). In general, the frequencies at which V_H gene families are productively rearranged in adult mice is stoichiometric, that is, proportional to V_H family size (1–3). However, in fetal or neonatal mice V_H expression is biased for those V_H exons located in the 3′ region of the locus (2). In contrast, neither is the expression of V_κ gene families in the adult mouse stoichiometric nor is there bias for the use of 3′ exons in the neonate (5–7).

Here we address the combinatorics of specific V_H and V_κ family pairings by determining the frequencies at which several V_H and V_κ families are expressed both independently and in combination. Two experimental strategies were employed. First, the filter paper disc method for lymphocyte cloning was used to screen large numbers of lipopolysaccharide (LPS)-induced B-cell colonies for V_H and V_κ expression (8). Sequential hybridizations of single discs permitted identification of C57BL/6-derived B-cell clones expressing specific V_H + V_κ family pairings (9). Second, two panels of hybridomas, derived from LPS-activated BALB/c or NZB splenocytes, were created by selection of clones positive for expression of the X-24 V_H family (BALB/c) or expression of the V_κ1 gene family (NZB). Subsequently, all clones in each panel were retyped for either V_κ or V_H expression. The results of both experimental strategies support the conclusion that V_H and V_κ families pair without bias. Thus, if some V_H family X is expressed among B cells at frequency x, and a V_κ family Y at frequency y, the measured frequency of B cells expressing both (X + Y) families is not significantly different from the product ($x \cdot y$). These experiments constitute direct genetic evidence that *in vivo* the recombinational choices at one *Ig* locus do not affect those at another.

Should unbiased pairing of V_H and V_κ families prove true generally, the frequency of any V_H + V_κ pairing may be calculated whenever the independent V_H and V_κ frequencies are known. We have done this for 9 V_H and 12 V_κ gene families to predict the frequencies at which specific V_H + V_κ family pairings are expressed in neonatal and adult C57BL/6 mice. Such expected frequencies are in excellent agreement with actual, observed frequencies. The distributions of expected frequencies are nonhomogeneous and change dramatically with the maturation of the mouse, illustrating a patterned acquisition of antibody diversity.

MATERIALS AND METHODS

Mice. C57BL/6, BALB/c, and NZB mice were purchased from The Jackson Laboratory and maintained locally. Donors of splenocytes for disc cultures were age-matched (≤7 days or 16–24 weeks) female C57BL/6 mice; thymocyte feeder cells were obtained from 5- to 8-week-old C57BL/6 mice. Hybridomas were derived from 4- to 5-week-old BALB/c or 3- to 6-month-old NZB mice.

Media and Mitogen. Splenocytes were cultured on filter paper discs in supplemented RPMI-1640 medium (8). Growth and selection media for hybridomas were those of Galfré *et al.* (10). *Escherichia coli* LPS was prepared (8) and used at ≈20 μg/ml to stimulate blastogenesis.

DNA Probes. DNA hybridization probes specific for V_κ gene families (V_κ1, -2, -4, -8, -9, -10, -19, -21, -22, -23, -24, or -28) (11–13), V_H gene families (V_H7183, -Q52, -S107, -X-24, -36-60, -VGAM 3-8, -J606, -3609, or -J558) (9, 14), or the μ constant (C_μ) region (3) have been described. Routinely, probes were ^{32}P-labeled by nick-translation.

Disc Cultures. Coordinate expression of specific V_H and V_κ gene families was determined in C57BL/6 mice by sequential hybridizations of LPS-induced colonies of splenic B cells (9). Particular V_H + V_κ pairings were identified in single colonies by three rounds of hybridization and autoradiography: first with ^{32}P-labeled probes specific for the X-24, S107, or Q52 V_H families (V_H^+); second with probes specific for the V_κ1 or -8 gene families (V_κ^+); and finally with a probe specific for the C_μ region (C_μ^+). Between successive hybridizations ≥95% of bound ^{32}P was stripped by stringent washing (3). Frequencies of independent V_H and V_κ gene family expression were

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Abbreviations: V, variable; C, constant; LPS, lipopolysaccharide.

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determined as (no. of V_H^+ or V_κ^+ colonies per disc) ÷ (no. of C_μ^+ colonies per disc). Frequencies at which specific V_H + V_κ family pairings were expressed were determined as (no. of V_H^+ and V_κ^+ colonies per disc) ÷ (no. of C_μ^+ colonies per disc).

Production of Hybridomas. Splenocytes from BALB/c or NZB mice were cultured for 2 days in medium containing LPS (25 μg/ml) and then fused to SP2/0-Ag14 cells by use of polyethylene glycol (10).

Selection of Hybridomas Expressing V_HX-24 or V_κ1 Exons. The method of Manser and Gefter (15) was used to identify BALB/c-derived hybridomas expressing the V_HX-24 gene family and NZB-derived hybridomas expressing the V_κ1 gene family, as described (16, 17). All positive BALB/c lines and 18 randomly selected NZB lines were cloned by limiting dilution and rescreened under high-stringency conditions.

Northern Blotting. Total RNA was extracted (18), electrophoresed through a 1.2% agarose/formaldehyde gel, transferred to nitrocellulose, and hybridized with V_H- or V_κ-specific probes followed by stringent washing (19). RNA blots from BALB/c-derived X-24$^+$ clones were hybridized with V_κ1, -2, -4, -8, -9, -10, -19, -21, -22, -23, -24, and -28 probes while blots from NZB-derived $V_\kappa 1^+$ clones were hybridized with probes specific for the V_H7183, -Q52, -S107, -X-24, -36-60, -3609, -VGAM 3-8, -J606, and -J558 gene families. No cross-hybridization among the different V_H and V_κ probes used was seen (Fig. 1).

RESULTS

Independent and coordinate expression of V_H and V_κ gene families was determined in C57BL/6 mice by sequential hybridizations of LPS-induced colonies of splenic B cells (3, 5). Colonies expressing particular V_H + V_κ family pairings were identified as congruent colonies in serial V_H-, V_κ-, and C_μ-specific hybridizations. Among some 4.1×10^4 C_μ^+ colonies, ≈2.2×10^3 and ≈8.6×10^3 expressed one of the V_H or V_κ families studied, respectively (Table 1). In agreement with our previous work (3, 9), the V_HX-24, -S107, and -Q52 families were expressed by about 1%, 2%, and 12% of colonies. In the neonate, V_HQ52 exons were expressed by almost 19% of colonies, confirming other reports (2, 4). Likewise, the V_κ1 and -8 gene families were expressed in adult mice at frequencies of about 25% and 14%, respectively. As reported (5), V_κ1 exons dominated light-chain expression in the neonate; some 39% of such colonies used V_κ1 family exons.

If the recombinational choices at the expressed heavy- and light-chain loci are independent, the frequencies of particular V_H + V_κ family pairs may be calculated from the independent V_H and V_κ frequencies. For example, in Table 1 neonatal mice are shown to express V_HQ52 and V_κ1 exons among 18.93% and 38.53% of C_μ^+ colonies. If Q52 and V_κ1 exons pair randomly, the predicted frequency of coordinate V_HQ52 and V_κ1 expression is simply 18.93% × 38.53% = 7.29%. Among the 2240 C_μ^+ colonies screened in this sample, the expected number of colonies expressing both Q52 and V_κ1 exons is 2240 × 0.0729 ≈ 163. Similar calculations have been performed for each of the V_H + V_κ pairings investigated so that the number of family pairs observed may be compared with the number expected. Thus, the 163 V_HQ52$^+V_\kappa 1^+$ colonies expected among C_μ^+ colonies derived from neonatal mice should be compared to actual numbers of such colonies observed in serial hybridizations, 170. In all cases, the number of colonies expected and observed to express par-

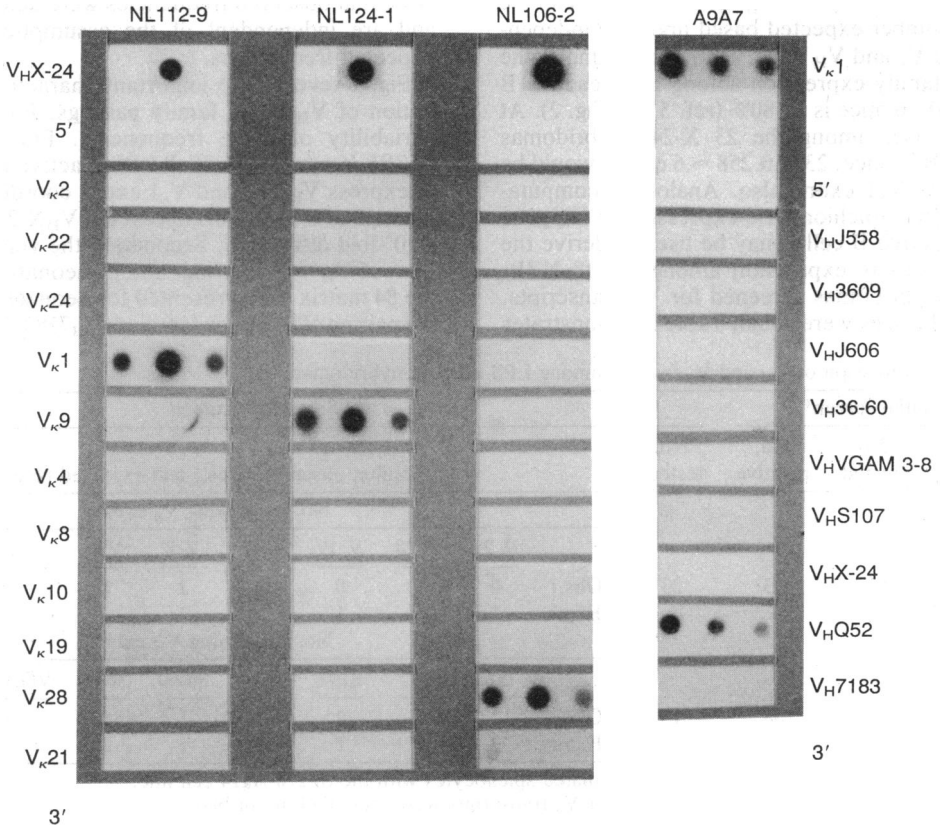

FIG. 1. Controls for specificity of V_H and V_κ probes by dot blot hybridization. (*Right*) Total RNA taken from the NZB-derived $V_\kappa 1^+$ hybridoma clone A9A7 was hybridized with the 9 V_H family-specific DNA probes illustrated. Only V_HQ52-specific hybridization was observed. (*Left*) Total RNA taken from three BALB/c-derived V_HX-24$^+$ hybridoma clones (NL112-9, NL124-1, and NL106-2) was hybridized with the 11 V_κ family-specific DNA probes illustrated. Lack of cross-hybridization among the probes employed permits unambiguous assignment of V_H and V_κ expression to hybridomas and colonies.

Table 1. Comparison of expected versus observed numbers of B-cell colonies expressing specific V_H + V_κ pairs

Age*	No. of discs	V_H probe specificity	No. V_H^+	V_κ probe specificity	No. V_κ^+	No. C_μ^+	V_H/C_μ, %	V_κ/C_μ, %	No. $V_H^+V_\kappa^+$ Expected†	No. $V_H^+V_\kappa^+$ Observed‡
Neonate	5	Q52	424	V_κ1	863	2,240	18.93	38.53	163	170
Adult	4	X-24	79	V_κ1	2180	7,564	1.04	28.82	23	22
	4	X-24	104	V_κ8	1183	8,402	1.24	14.08	15	14
	3	S107	145	V_κ1	1135	5,966	2.43	19.02	28	34
	3	S107	109	V_κ8	727	5,419	2.01	13.42	15	18
	3	Q52	693	V_κ1	1565	5,967	11.61	26.23	182	147
	3	Q52	657	V_κ8	908	5,772	11.38	15.73	103	84
	25		2211		8561	41,330			529	489

*Female C57BL/6 mice were ≤7 days (neonate) or ≥16 weeks (adult) when used as cell sources.
†Calculated as % V_H × % V_κ × no. C_μ^+.
‡Determined as congruent labeled colonies in sequential hybridizations.

ticular V_H + V_κ pairings is in good agreement, consistent with a process of unbiased association.

To substantiate the generality of unbiased V_H + V_κ family pairing, 1740 hybridoma lines were prepared from LPS-stimulated splenic lymphocytes taken from BALB/c or NZB mice. The 912 hybridoma lines derived from BALB/c mice were screened for expression of the V_HX-24 gene family; 23 positive clones were identified, yielding a frequency of 2.53% (Table 2). RNA extracted from these X-24$^+$ hybridomas was then hybridized in Northern blots with probes specific for different V_κ families. In contrast, the 828 hybridoma clones derived from NZB mice were initially screened for expression of the V_κ1 gene family (Table 2). Subsequently, of the 88 $V_\kappa1^+$ hybridomas detected (10.63%), 18 were chosen randomly and cloned for screening with probes specific for one of nine V_H families. As for the analysis of B-cell colonies, the number of hybridomas positive in secondary screenings for transcripts of particular V_κ or V_H gene families can be compared to the number expected based upon the independent frequencies of V_κ and V_H expression. For example, the frequency of V_κ1 family expression among LPS-reactive B cells in adult C57BL/6 mice is 25.80% (ref. 5 and Fig. 2). At this frequency of use, among the 23 X-24$^+$ hybridomas derived from BALB/c mice, 23 × 0.258 ≈ 6 clones would be expected to express V_κ1 exons also. Analogous computations, based upon the stoichiometric expression of V_H gene families in LPS-reactive B cells, may be used to derive the distribution of V_H family expression among the 18 NZB-derived (4), $V_\kappa1^+$ hybridomas screened for V_H transcripts. Although the sample sizes were small, Table 2 demonstrates significant correlations between the number of observed versus expected V_H + V_κ family pairings ($P \leq 0.05$).

In general, unbiased association of V_H and V_κ families permits calculation of the expected frequency of any V_H + V_κ pairing given that the independent V_H and V_κ frequencies are known. The frequency matrices shown in Fig. 2 depict the expected usage for 84 V_H + V_κ family pairings in neonatal and 108 V_H + V_κ pairings in adult C57BL/6 mice. Marginal frequencies (outside the matrix) indicate the independent frequencies at which specific V_H or V_κ gene families are used. Values within matrix cells estimate the frequency of specific V_H + V_κ pairs. Cells depicted in bold outline contain both the expected (upper) and observed (lower; from Table 1) frequencies. Despite the several assumptions—most notably, stoichiometric V_H expression—used to generate Fig. 2, concordance between expected and observed values is excellent, well within the variance expected of a Poisson process (23). Note that observed frequencies were determined empirically and are independent of the assumptions used to derive expected frequencies.

Fig. 2 reveals two important characteristics of the distribution of V_H + V_κ family pairings. First is the significant variability of these frequencies. For example, in adult C57BL/6 mice, 12% of all LPS-reactive B cells are expected to express V_HJ558 and V_κ1 exons coordinately. In contrast, <0.01% of B cells will express the V_HX-24 and V_κ24 families, a 10^3-fold difference. Second are the very different distributions of V_H + V_κ pairings among neonatal and adult mice. Of the 84 matrix cells presented for neonates, 6 cells formed by the pairing of five gene families (V_H7183, V_HQ52, V_HJ558 and

Table 2. Coordinate expression of V_H and V_κ families among LPS-induced hybridomas

Source of hybridoma clones	Initial screen* Probe	No. tested	No. positive	Secondary screen No. tested†	Results, observed (Obs.) and expected (Exp.)‡									
						No. expressing V_κ exons§								
						V_κ21	V_κ28	V_κ19	V_κ10	V_κ8	V_κ4	V_κ9	V_κ1	V_κ24
BALB/c	V_HX-24	912	23	23	(Obs.)	0	1	0	0	1	0	1	8	0
					(Exp.)	1	0	1	0	2	1	1	6	0
						No. expressing V_H exons¶								
						7183	Q52	S107	X-24	36-60	3609	VGAM3-8	J606	J558
NZB	V_κ1	828	88	18	(Obs.)	4	2	0	0	1	2	0	0	5
					(Exp.)	2	2	1	0	1	2	1	1	8

Hybridomas were produced by the fusion of LPS-stimulated splenocytes with the SP2/0-Ag14 cell line.
*RNA was recovered from cell lysates and specific V_H or V_κ transcripts were identified in dot blots.
†All dot blot results were verified in Northern blots.
‡Expected values are based upon stoichiometric expression of V_H families (see ref. 20 for complexities) or measured frequencies of V_κ expression (5). In both comparisons, the observed and expected distributions of V_H or V_κ expression do not differ significantly [BALB/c, $\chi^2 = 13.64$; NZB, $\chi^2 = 7.66$ (d.f. = 8) $P \leq 0.05$].
§Among the 23 BALB/c-derived hybridomas screened, V_κ-family expression was not identified in 12 lines.
¶Among the 18 NZB-derived hybridomas screened, V_H-family expression was not identified in 4 lines.

Neonate

Cμ JH DH	7183	Q52	S107	X-24	36-60	J606	J558	
.0017	.0004	.0003	.0001	<.0001	.0001	.0001	.0003	V_κ 22
.0474	.0119	.0090	.0014	.0005	.0028	.0014	.0085	V_κ 2
.0003	.0001	.0001	<.0001	<.0001	<.0001	<.0001	.0001	V_κ 24
.4005	.1001	.0758 (.0759)	.0120	.0040	.0240	.0120	.0721	V_κ 1
.2297	.0574	.0435	.0069	.0023	.0138	.0069	.0413	V_κ 9
.0540	.0135	.0102	.0016	.0005	.0032	.0016	.0097	V_κ 4
.1380	.0345	.0261	.0041	.0014	.0083	.0041	.0248	V_κ 8
.0033	.0008	.0006	.0001	<.0001	.0002	.0001	.0006	V_κ 10
.0120	.0030	.0023	.0004	.0001	.0007	.0004	.0022	V_κ 19
.0049	.0012	.0009	.0001	<.0001	.0003	.0001	.0009	V_κ 28
.0577	.0144	.0109	.0017	.0006	.0035	.0017	.0104	V_κ 23
.0002	.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	V_κ 21
	.25	.1893	.03	.01	.06	.03	.18	J_κ
								C_κ

Adult

7183	Q52	S107	X-24	36-60	VGAM 3-8	J606	3609	J558	
.0040	.0051	.0013	.0007	.0017	.0017	.0034	.0051	.0202	.0431
.0015	.0019	.0005	.0003	.0006	.0006	.0013	.0019	.0077	.0165
.0002	.0003	.0001	<.0001	.0001	.0001	.0002	.0003	.0012	.0026
.0242	.0302 (.0305)	.0081 (.0046)	.0040 (.0030)	.0101	.0101	.0201	.0302	.1210	.2580
.0048	.0060	.0016	.0008	.0020	.0020	.0040	.0060	.0239	.0509
.0031	.0038	.0010	.0005	.0013	.0013	.0026	.0038	.0153	.0327
.0084	.0105 (.0179)	.0028 (.0027)	.0014 (.0013)	.0035	.0035	.0070	.0105	.0421	.0899
.0013	.0016	.0004	.0002	.0005	.0005	.0011	.0016	.0063	.0135
.0057	.0071	.0019	.0010	.0024	.0024	.0048	.0071	.0285	.0609
.0004	.0005	.0001	.0001	.0002	.0002	.0003	.0005	.0019	.0040
.0023	.0029	.0008	.0004	.0010	.0010	.0019	.0029	.0116	.0247
.0024	.0030	.0008	.0004	.0010	.0010	.0020	.0030	.0121	.0259
.0938	.1172 (.1252)	.0313 (.0266)	.0156 (.0197)	.0391	.0391	.0781	.1172	.4688 (.4524)	

FIG. 2. Expected distribution of V_H + V_κ family pairings in neonatal and adult C57BL/6 mice. Values within matrix cells estimate frequencies of V_H + V_κ pairings. Cells in bold outline also contain observed frequencies (in parentheses). Marginal frequencies indicate independent frequencies at which V_H or V_κ gene families are used. Independent V_κ frequencies have been determined by colony hybridizations (ref. 5 and Table 1) and represent the census of $\approx 8.8 \times 10^4$ C_μ^+ colonies. V_H expression in adult mice is based upon the complexity of each family (20). Observed frequencies for the V_HQ52, -S107, -X-24, and -J558 families (refs. 3 and 9; Table 1) are also given (in parentheses) and represent the census of $\approx 1.2 \times 10^5$ C_μ^+ colonies. Except for V_HQ52, frequencies for V_H expression in the neonate are averages taken from refs. 4 and 21. The V_HQ52 frequency is from Table 2. V_H and V_κ gene order is based on refs. 22 and 13, respectively. The V_κ2 and -22 families are not mapped.

V_κ1, V_κ9) represent expected frequencies of 10% to >4%. Together, these 6 V_H + V_κ pairings account for nearly 40% of all LPS-reactive B cells in the neonatal spleen. The distribution of V_H + V_κ associations is more disperse in adult mice. Only 2 (V_HJ558 and V_κ1, V_κ8) of the 108 family pairings studied in adults represent expected frequencies >4%. Finally, although interspersion of V_H families has been demonstrated (22, 24) and is expected for V_κ (13, 25), the axes of the matrices correspond to the known ordering of gene families within the *Igh* and *Igκ* loci (13, 22, 25). Thus, recombinational biases that may be developmentally regulated (2) can be followed in two dimensions.

DISCUSSION

Prior to encounter with antigen, the primary antibody repertoire is largely determined by two processes, (*i*) rearrangement of the heavy- and light-chain V-region genetic elements and (*ii*) the rates at which heavy and light chains pair to form specific associations (26, 27). In studies of the first of these two processes, we and others have determined frequencies at which V_H and V_κ gene families are expressed in neonatal and adult mice. In this study, among 4.1×10^4 C_μ^+ colonies, expression of the X-24, S107, and Q52 V_H families was stoichiometric in adult mice while in neonates expression of V_HQ52 exons was substantially increased (Table 1). The present data confirm our earlier study of V_κ expression in neonatal and adult mice (5) and extend these findings by reporting frequencies at which the V_κ23 and V_κ28 gene families are expressed (Fig. 2). Interestingly, LPS-induced hybridomas derived from NZB mice expressed V_κ1 exons at a frequency of $\approx$11% (Table 2), about half that for C57BL/6 or BALB/c (5, 6). As the *Igκ* haplotypes of these strains differ [NZB, $Ig\kappa^b$; BALB/c and C57BL/6, $Ig\kappa^c$ (25)], we believe that this difference represents haplotype-dependent control of V_κ expression.

The purpose of this study was to address the second process of repertoire formation by investigating rates at which different V_H and V_κ gene families combine. Using two independent experimental strategies, *in situ* hybridizations of B-cell colonies and Northern blot analysis of mitogen-induced hybridomas, we determined frequencies for a total of 25 V_H + V_κ family pairings in C57BL/6, BALB/c, and NZB mice (Tables 1 and 2). Results from both kinds of experiments were concordant. Serial hybridization of C57BL/6-derived B-cell colonies revealed that coordinate expression of the V_HX-24, -S107, or -Q52 families and V_κ1 and V_κ8 exons appears to be unbiased. Thus, observed frequencies for specific V_H + V_κ pairings could be approximated by the product of the independent V_H and V_κ frequencies (Table 1). Similarly, when panels of V_HX-24$^+$ BALB/c-derived and V_κ1$^+$ NZB-derived hybridomas were studied for coexpression of nine V_κ or V_H gene families, the patterns of observed and expected pairings were not statistically different (Table 2). Although this study did not determine the frequencies for all possible V_H + V_κ pairings, our data indicate that V_H and V_κ gene families may be said to pair randomly. This remains true even when the independent V_κ frequencies are altered in a strain-dependent fashion ($Ig\kappa^b$ versus $Ig\kappa^c$). V_H recombination and expression do not affect the subsequent selection of V_κ exons.

While the two methods used in this study to define V_H + V_κ frequencies are very different, both techniques rely upon

the use of LPS to activate B cells. For this reason, it may not be appropriate to extend our conclusions to B cells insensitive to this mitogen. However, Jeong and Teale (28) have demonstrated comparable V_H expression among resting and LPS-activated B cells. Thus, we believe that the population of LPS-sensitive splenic B lymphocytes offers an appropriate sample for analyses of V_H and V_L expression.

V_κ gene families are characterized by substantially greater interfamily homologies than are V_H families (25). Thus, studies of V_κ expression based upon the use of family-specific probes are sensitive to hybridization conditions. For example, Teale and Morris (6) have reported V_κ use that generally substantiated our findings (5). Lawler *et al.* (7) published similar results based upon analysis of hybridomas. However, all reports differ in the details of V_κ expression. More recently, screening of splenic cDNA libraries with V_κ family-specific probes has yielded frequencies validating those determined by *in situ* hybridization (refs. 5 and 6; P. Brodeur, personal communication).

Finally, inspection of $V_H + V_\kappa$ frequency distributions (Fig. 2) illustrates that the antibody repertoire, approximated by $V_H + V_\kappa$ family pairings, appears to be distributed in a distinctly nonuniform pattern. Certain $V_H + V_\kappa$ pairings exist at high frequencies while others are exceedingly rare. Even though this patchy distribution is most noticeable in the neonate, it extends into adult life. The sources of this substantial variability are differences in V_H family size and biased use of V_H and V_κ gene families. In the neonate where expression of both V_H and V_κ families is strongly biased, a cluster of only six $V_H + V_\kappa$ pairings accounts for almost 40% of the antibody repertoire. In adult mice, expression of V_H families is stoichiometric (3, 9) and it is the biased use of V_κ exons (5) that determines the distribution of $V_H + V_\kappa$ pairings. The result of nonuniformity in the distribution of $V_H + V_\kappa$ family pairs is to limit and perhaps direct the diversity of the antibody repertoire. The significance of this shaping of the repertoire remains to be explored, yet the somatic genetic events we describe coincide with a patterned acquisition of humoral immune responsiveness that has been documented in the developing fetus and neonate of sheep (29), opossum (30), mouse (31), and chicken (32). These orderly events may be significant in establishing and diversifying the immune system.

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