DIFFERENTIAL EXPRESSION OF SETS OF HIGHLY HOMOLOGOUS VARIABLE REGION GENE PRODUCTS IN SELECTED AND PREIMMUNE REPERTOIRES OF INBRED MOUSE STRAINS

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The antibody repertoire is determined by multiple joining gene segments (J_k, J_λ, J_H, and D_H) and by multiple heavy and light chain variable regions (V_H and V_L) (1–3).

The V_H and V_k loci of the mouse are currently estimated to consist of few hundred genes (4, 5). These genes fall into families defined by the homology of their nucleic acid sequences (6, 7), and the size of these families varies from >60 genes (the V_H-J558 family) (5) to just one ($V_\lambda 1$ and $V_\lambda 2$) (8).

Classical works have also defined certain germ line H and L chain V region genes that are selectively used by certain, but not other, strains of mice in particular immune responses (9–11). Although the overall complexity of each V_H family is highly conserved (5), restriction fragment analysis has also revealed extensive polymorphism of each V_H gene family (5). Such a polymorphism has also been recently described for V_k gene subgroups (12–18).

Little is known, however, about whether this polymorphism affects the frequency to which each V_H and V_k gene segment is available in the preimmune repertoire.

This analysis clearly requires reagents that can recognize all the allelic forms of a given V_H or V_k gene product independently from its property to impart antibody activity to the Ig in which it is used.

We sought to approach this problem by analyzing the repertoire of various mice strains with two mAb that respectively recognize the V_k21D-E L chain (19–21) subtypes and the V_HT15 gene segment product of all inbred mouse strains (22). This analysis has allowed us to (*a*) define the level of expression of these gene's products in the normal sera of a large collection of inbred mice, (*b*) obtain a view of the probability of expression of the various allelic forms of these gene products by estimating the frequencies of B cells that use either the V_HT15 and V_k21D and -E segments before antigenic encounter, and (*c*) determine the

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influence of the various polymorphic forms of the V_HT15 gene segment on the specificity of the Ig to which it is associated.

Materials and Methods

Mice and Mitogen. All inbred strains of mice used in this work were obtained from the colony of the Institut Pasteur. Pooled sera from NX8 recombinant inbred (RI)[1] (23) strains were kindly provided by Dr. R. Riblet (Institute for Cancer Research, Philadelphia, PA).

LPS from *S. typhimurium* was obtained from Difco Laboratories (Detroit, MI).

Culture Conditions for Frequency Analysis. Frequency analysis was performed with LPS as described by Anderson et al. (24). Spleen cell suspensions were cultured in RPMI 1640 supplemented with glutamin, antibiotics, 10% FCS, and 2×10^{-5} M 2-ME in the presence of rat thymus filler cells at a concentration of 3×10^6 cells/ml. At each cell dose, 24 replicate cultures were set up and their supernatants were tested after 8 d of culturing.

Cells and Proteins. The following hybridomas and their purified Ig products were used. H106-131 monoclonal anti-V_k21D-E was a kind gift of Dr. M. Pierres (Marseille, France) and obtained as previously described (19). The TC54 anti-V_HT15 mAb was kindly provided by Professor M. Scharff, and has been previously described (22). MOPC511, HOPC8, TEPC-15, MOPC167, and HOPC603 cell lines were obtained from Dr. M. Potter, and their proteins were isolated from ascitic fluid.

The 14.4.4. (V_k21 E) anti-Ia-7 mAb (25) was a kind gift of Dr. D. Sachs (National Institutes of Health, Bethesda, MD) and isolated on protein A–Sepharose.

The rat anti–mouse κ mAb H106-52-1 was kindly provided by Dr. M. Pierres.

Ascitic fluids from 199 BALB/c myelomas were kindly provided by Dr. M. Potter.

Isolation of V_HT15^+ Ig. Mice were bled from the retroorbital plexus, and sera were precipitated with 18% Na_2SO_4. V_HT15 Ig were thereafter isolated on TC54-Sepharose AH.

Radioimmunoassays. Two types of RIA were used in these studies. The first one consisted of a radioactive binding inhibition, and was used to quantify the levels of V_HT15 and V_k21 Ig in sera as well as the frequencies of their expression in LPS-sensitive cells. Briefly, serial sera dilutions or cultures supernatants were added to 14.4.4. (V_k21E) (1 μg/ml), M511 (1 μg/ml), or F6(51) (γ1, k) (1 μg/ml) –precoated plastic wells together with ^{125}I-labelled H106-131, TC54, or H106-52-1 antibody. After an overnight incubation, the plates were washed, and bound radioactivity was measured in a gamma counter.

The second assay consisted of a direct binding test. To determine the frequencies of V_HT15 or V_k21D-E^+ clones, as well as of those with anti-PC activity, cultures supernatants were added to H106-131 (3 μg/ml), TC54 (3 μg/ml), or PC-BSA (10 μg/ml) –precoated plastic wells. The plates were incubated overnight at 4°C, washed, and reincubated for 6 h respectively with ^{125}I–H106-131, -TC54, or –anti-k mAb H139-52-1.

Total, as well as V_HT15^+ and V_k21D-E^+ anti-PC antibodies, were detected by adding serial dilutions of sera to PC-SAB–coated plates for 12 h at 4°C. Bound molecules were thereafter detected by reincubating the plates with ^{125}I-TC54, –H106-131, or –H106-52-1.

Frequency Determinations. Supernatants were considered positive when the percentage of inhibition or the number of bound cpm exceeded by three SD the mean value of control cultures containing LPS and filler cells. Minimal estimates of precursor frequencies were obtained by the regression analysis from the Poisson distribution relationship between the responding cell number and the logarithm of the percentage of nonresponding (negative) cultures. Absolute frequencies were determined by dividing the observed precursor frequency by the frequency of LPS-responding cells.

Results

The properties and the fine specificity of the H106-131 anti-V_k21D-E subgroup have been previously described (18). The anti-V_HT15 mAb TC54 was

[1] *Abbreviation used in this paper:* RI, recombinant inbred.

TABLE I

Levels of V_HT15 and V_k21 D-E Gene Products in Normal Sera of Inbred Mouse Strains

Strains	H-2	Igh-V*	Igh-C	V_HT15 Ig (μg/ml)	V_k21 D-E Ig (μg/ml)
BALB/c	d	a	a	51	202
BALB.B	b	a	a	36	383
BALB.K	k	a	a	29	360
C.B20	d	b	b	17	312
BAB.14	d	a	b	112	1200
RIII	r	a	j	10	500
C58	k	a	a	28	100
C57BL/6	b	b	b	0.7	312
C57BL/6 H-2^k	k	b	b	28	350
C57BL/6 Igh^a	b	a	a	82	315
SJA	s	a	a	28	314
SJL	s	b	b	8.4	500
PL	u	j	j	28	400
LP	b	b	b	28	900
NZB	d	j/d	n	0.3	900
C3H	k	j	j	13	450
AKR	k	j/d	d	0.9	350

The values are expressed as 14.4.4. (V_k21E) and MOPC T15 (V_HT15) equivalent and determined by the ability of pooled sera from each strain to inhibit the H106-131 14.4.4. and MOPC511-TC54 interactions.

* According to Brodeur and Riblet (5).

obtained by immunizing rats with S107 protein (22). The TC54 molecule has been shown to react with free T15 H chains and with T15 H chains associated with irrelevant chains. Within the V_H-4 subgroup, TC54 recognizes the V_H gene segment of TEPC 15, S107, HOPC-8, MOPC-511, MOPC-167, but not the one of Mc-603 (22). The lack of reactivity to McPC-603 is believed to be due to conformational changes caused by the two–amino acid differences in the second hypervariable region, or to the four substitutions in the third hypervariable region, which distinguish it from the T15-S107 H chain V region (22). The selective specificity for V_HT15 of the TC54 antibody was further tested by studying the property of 199 BALB/c myeloma proteins, representative of all the V_H isotypes known, to inhibit the binding of TC54 to HOPC-8. Only 5 out of the 199 proteins were inhibitory (data not shown). Three of these proteins were MOPC-511, MOPC-167, and TEPC-15, while the other two had V_H not yet sequenced.

Levels of V_HT15 and V_k21D-E in Normal Sera of Inbred Mice. The availability of mAb specific for V_H and V_k genes products offers a unique opportunity to study the level of expression of these genes segments independently from the antibody specificity of the Igs to which they are used.

In the first series of experiments, we quantified the level of these gene segments' products in the sera of several inbred mouse strains (Table I). Initially, our main concern was that the two reagents would not recognize all the allotypic forms of the V gene segment under study. This is apparently not the case, as all sera studied contained molecules capable of inhibiting both the V_HT15-TC54

TABLE II

Level of V_HT15 Ig in Normal Sera of NX8 Recombinant Inbred Strains

Strain	H-2	Igh-1	Igh-Dex	V_HT15^+ Ig (μg/ml)*
C58	k	a	a	26
NZB	d	n	j	0.4
NX8 4	8	8	8	21
NX8 9	N	8	8	19
NX8 13	8	8	8	26
NX8 15	N	N	N	24
NX8 16	8	8	8	16
NX8 18	N	8	8	15
NX8 20	N	8	8	19

H-2, Igh-1, and Igh-Dex are according to Riblet et al. (23). The table entries have the following meanings: 8 indicate that the particular strain has inherited the C58 allele at the designed locus; N indicates inheritance of the NZB allele.

* The level of V_HT15 was determined in pooled sera of the different strains.

and the 14.4.4.-H106-131 interactions, and all sera inhibited these interactions to generate curves with similar slopes (data not shown).

All the sera studied contained higher levels of V_k21D-E molecules than V_HT15^+ Ig. The ratio of V_k21D-E to V_HT15 varied from a minimum of 4 in BALB/c serum to a maximum of 3,000 in the sera of NZB animals. These exceedingly high variations are mainly due to the content of V_HT15^+ molecules, which can vary over a range of 300-fold from one strain to another; i.e., BAB.14 and NZB. Although, as a general rule, we could not detect any clear linkage between the serum level of V_HT15^+ and V_k21D-E^+ molecules to loci linked either to H-2 or to Igh, we further investigated the abnormally low level of V_HT15 molecules observed in the sera of NZB mice by analyzing the quantity of these molecules in the normal sera of seven NX8 recombinant strains (23). Table II shows that the level of V_HT15^+ molecules in the NX8 RI strains tested, including NX8 15, was similar to that of C58. Thus, unless a recombination has occurred in the Igh-V region of the NX8 15 strain, we must conclude that the abnormally low level of V_HT15 gene segments in the normal serum of NZB animals cannot be traced to the allelic form of the coding gene.

Frequencies of V_HT15^+ and V_k21D-E^+ B Cells in Preimmune Repertoire. The serological data presented above reflects a repertoire of Igs selected by internal and environmental antigens. Consequently, they do not provide direct information, concerning the mechanism responsible for the dominance of V_k21D-E over V_HT15, or for the influence of genetic polymorphism on V_H expression. To begin to understand these processes, it is necessary to have an understanding of the frequency of expression of defined V_H and V_k genes in the preimmune B cell population. The usual characterization of preimmune B cell repertoire involves the analysis of antibodies secreted by mitogenically stimulated B cells. Although it may be questioned whether the complete repertoire of antibody specificities is present among mitogen-reactive B cell subsets (26–30), this methodology re-

TABLE III

Frequencies of V_HT15 and V_k21 D-E Segments Used in Preimmune B Cell Clones

Strain	Frequency* of LPS-sensitive cells	Absolute frequencies* of clones:		
		V_HT15	V_k21	Anti-PC
BALB/c	20	160	22	50
BALB.B	22	283	30	60
BALB.K	19	253	26	69
C.B20	26	1,600	20	60
C3H	6	1,100	25	55
C57BL/6	6	1,300	28	80
C57BL/6 H-2^k	8	800	30	50
C57BL/6 Igh^a	5	100	22	60
SJL	30	1,600	31	90
C58	4	380	29	100
A/J	15	600	22	70

Frequencies determined as described in Materials and Methods. Absolute frequencies were determined by dividing the observed frequencies by the frequencies of LPS-sensitive cells.

* Frequencies are expressed as reciprocals.

mains, at the present time, the best available one for the analysis of the unselected B cell repertoire. Thus we determined, by limiting-dilution analysis, the frequency of B cells of several mice strains producing $V_k21D\text{-}E^+$ and V_HT15^+ Ig in response to LPS. As V_HT15 is associated with the immune response to PC (9, 31–33), we found it also interesting to correlate the number of V_HT15 precursors to the one of B cell clones with PC activity. All values were corrected for the frequency of LPS-reactive cells in the spleen of the various strains tested. As shown in Table III, the frequency of B cells using V_HT15 varies from 1/110 in C57BL/6 Igh^a to 1/1,300 in C.B20. It is evident, from the results obtained with BALB/c and C.B20 as well as with C57BL/6 and C57BL/6 Igh^a that the polymorphism of the V_HT15 gene strongly influences its frequency of expression. It is also noteworthy that the ratio of V_HT15 to anti-PC–specific B cell precursor frequencies varies from a minimum of 1:26.7 in C.B20 mice to a maximum of 1:3.8 in the C58 strain. This observation shows that, even in BALB/c mice, a considerable proportion of clones using the V_HT15 segment are devoid of anti-PC activity, and also, they suggest that a large proportion of anti-PC clones in C.B20 mice do not use V_HT15.

Surprisingly, the frequency of V_HT15 in C58 mice was lower than the one of other mice with the V_HT15^a allelic form. Similarly, spleen cells of C57BL/6 H-2^k had a larger number of V_HT15^+ B cell precursors than those of C57BL/6. Although at the present time we do not have a clear cut explanation for these results, these observations may suggest that genes other that those encoding V_H may modulate the frequency of expression of V_H segments.

The frequency precursor analysis of B cells expressing the $V_k21D\text{-}E$ genes' products, on the other hand, gave a completely different picture than the one obtained with V_HT15. The number of $V_k21D\text{-}E^+$ precursors was, in effect, found

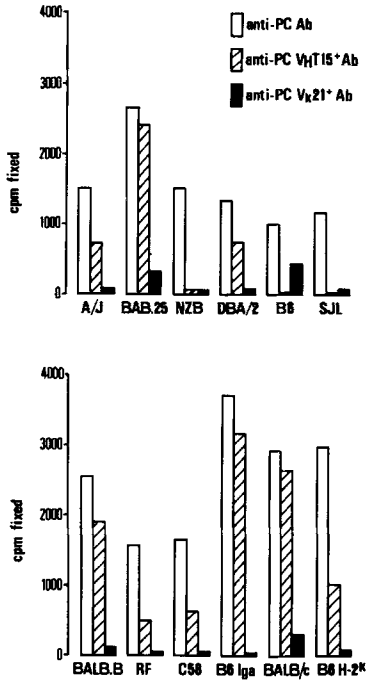

FIGURE 1. Analysis of the level of total (□), V_HT15^+ (▨), and $V_k21D\text{-}E^+$ (■) anti-PC antibodies in the normal serum of the indicated mouse strains. Pooled sera from five individual mice were tested at 1:100 dilutions for total and V_HT15^+ anti-PC activity, and at 1:10 dilution for V_k21^+ anti-PC activity.

to be remarkably similar, if not identical, in the splenocytes of all the strains tested. This was the case also for C58 and SJL mice, which have recently been shown (18) to express two different allelic forms of the V_k21 genes from the majority of all other laboratory mouse strains. This observation, together with the findings that, even in the preimmune repertoire, the frequency of $V_k21D\text{-}E$ gene segment expression was very high, and in all cases more important than that of V_HT15, suggests either a nonrandom use of V_H genes or, alternatively, that the number of V_H genes is far larger than their V_k counterpart.

Influence of V_H Polymorphism on Antibody Specificity. To determine the contribution of the various polymorphic forms of V_HT15 to antibody specificity, we used two different strategies. The first consisted of analyzing the V_HT15^+ component among natural anti-PC antibodies in the sera of several mice strains. The result of these experiments are shown in Fig. 1. Although the data are not quantitative, they give a clear idea of the quality of the naturally occurring anti-PC antibody in the various animals tested. Three clear patterns could be distinguished. (*a*), BALB/c, BALB.B, BAB.25, and B6-Igh^a mice were characterized by molecules dominated by the presence of V_HT15. (*b*), Intermediate levels of V_HT15^+ component were present in the sera of A/J, DBA/2, C58, and C57BL/6 H-2^k animals. (*c*), Anti-PC antibodies that do not use the V_HT15 segments were found in the sera of NZB, C57BL/6, and SJL mice. Even in the sera of those

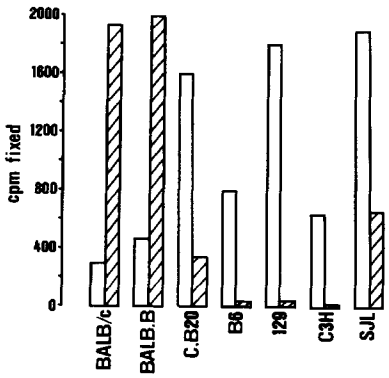

FIGURE 2. Analysis of the level of anti-PC antibodies in the V_HT15^+ (▨) and V_HT15^- (□) fractions of normal sera of the indicated mouse strains. V_HT15 Igs were isolated from the normal serum of the indicated strains on TC54-Sepharose AH and tested at a concentration of 4 μg/ml. The unbound fractions were concentrated at the original volume and tested at a final dilution of 1:50.

strains that do not use V_HT15 in the anti-PC antibody response, V_k21D-E gene segments were never found expressed on anti-PC antibody.

The second series of experiments consisted of isolating V_HT15^+ Igs from the normal sera of several strains using Sepharose-TC54, and then testing these molecules, as well as the unbound serum fraction, for anti-PC activity. The results, shown in Fig. 2, clearly show that, in BALB/c and BALB.B normal sera, the vast majority of anti-PC antibodies were recovered in the eluted fraction, while the same antibody activity was only present in the unbound component of C.B20, C57BL/6, C3H, and SJL sera. These results have been obtained with sera from nonimmunized mice, and therefore they should not be taken as evidence for the absence of V_HT15 usage by C57BL/6, C3H, C.B20, and SJL mice when artificially challenged with PC antigens. These data, however, establish that V_H polymorphism can profoundly influence not only the expression of a genetically defined set of idiotopes but also the overall capacity of a given Ig to react with a given epitope.

Discussion

The existence of V_H polymorphism in the mouse was initially identified in several systems; i.e., the demonstration of allelic forms of anti-PC antibodies, the absence from certain mouse strains of the 36–65 V_H gene (used in the A/J antiarsonate response) (34, 35), and by sequence differences in the NP and 36–60 genes in BALB/c, C57BL/6, and A/J mice (36). More recently, Brodeur and Riblet (5) have demonstrated by Southern blot analysis that this polymorphism is indeed extended to all known V_H families. Similarly polymorphism affecting V_k genes has also been reported (12–18).

We address the problem of the functional influence of V_H and V_k gene polymorphism on the expressed B cell repertoire. To this end, we used mAbs that recognize all the various alleles of the V_k21D-E and V_HT15 gene products. The gene products recognized by the reagents used in these studies may not be true alleles, but in fact may be pseudoalleles that may not occupy homologous

positions on paired chromosomes, and therefore exist as members of families of closely related structures.

Classical works on idiotypy (9–11) have defined certain germline H and L chain V region genes that are selectively used by certain, but not other strains of mice in response to well-defined antigens. Herein, we analyzed the overall probability of expression of all the allelic forms of a given V_H and a given V_k gene independently of antigen selection.

Our results show that the levels of V_HT15 fluctuate considerably in the sera of the different strains tested, and there was not direct correlation of the level of V_HT15^+ molecules and the Igh or the H-2 haplotype. Analysis of NX8 RI mice, moreover, excluded the possibility that the abnormally low level of V_HT15^+ Ig in NZB mice could be traced to the V_HT15 allele. The fact that V_H polymorphism is not responsible for the serum level of the corresponding protein is also documented by the observation that the level of V_HT15^+ molecules was >24 times higher in C.B20 than in C57BL/6. Thus, it must be concluded that genes other than H-2 and Igh play an important role in the selection of the available repertoire. The validity of this conclusion is further supported by our recent studies (37) on the polymorphism of V_k21D-E-associated H chains isolated from the normal serum of BXH recombinant mice. This analysis, in fact, revealed the existence of different isoelectric focusing spectrotypes from either parental strains in strains in which both H-2 and Igh-loci segregated from the same parent.

The level of V_k21D-E molecules always dominated over V_HT15, and this is apparently the case both in the normal serum and in the preimmune repertoire. The V_L gene pool is currently estimated to consist of about 100 genes (6). If one assumes that all V_k genes have an equal probability of expression, one should expect, in the preimmune repertoire, a frequency of 0.1% for any given V_k gene. Considering that the H106-131 mAb recognizes both V_k21D and V_k21E, the observed frequency of V_k21D-E precursors (0.3%) is highly compatible with this expected value. The possibility that all V_k genes have equal probability of expression is further supported by the remarkably redundant frequency of expression observed in the splenocytes of all the strain tested, and also by the fact that our estimate of the number of V_k21D-E^+ precursors is very similar to the one observed for B cells expressing the V_k6 gene segments (38).

The redundant frequency of V_k21 expression strongly contrasts with the fluctuation observed in the number of V_HT15^+ B cell precursors in various strains. Contrary to the data obtained at the serum level, the number of V_HT15^+ clones in the preimmune repertoire could be directly correlated to the allotypic form of the structural gene. Thus, V_HT15^a is 10 times more likely to be expressed than V_HT15^b. These differences are quite surprising, since Rudikoff and Potter (39) described a PC-binding myeloma protein of C57BL/6 origin with a V_H region 96% homologous to T15. The substitutions found at positions 14 and 16 were present in all induced C57BL/6 anti-PC antibodies, and therefore it is likely that these alternatives are encoded in the C57BL/6 germline, and consequently that this gene represents the C57BL/6 allelic form of the T15 V_H.

Although our frequencies were determined in the absence of antigens, it can still be possible that V_HT15 polymorphism may affect the ability of germline sequences to bind common environmental antigens. If this is the case, our results

could then be accounted for by selection by environmental antigens, which would asymmetrically expand, in different strains, the expression of certain V regions. We found this possibility very unlikely however, since Clafin and Davie (40, 41) showed that T15-type antibodies dominate the anti-PC response in most mice, and in spite of structural diversity among them, their site for hapten appears remarkably constant even among different strains. In addition to this, complete V region amino acid sequences of H and L chains of mAb have unequivocally shown that mice of each genotype use the same three V_k segments in combinations with the different allelic form of a single V_H gene to produce most anti-PC antibodies (42). Thus, the fluctuation observed in the number of V_HT15^+ clones cannot be easily accounted for by antigenic selection, and therefore, mechanistic alternatives to explain our findings should also be considered. One possible explanation for the results reported here could be traced to the specificity of our anti-V_H antibody, which recognizes both T15 and M167. Thus it could be argued that, in C57BL/6 mice, one of these two genes is not functional. This possibility, however, is very unlikely, as Rudikoff and Claflin (43) showed, by isoelectric focusing, that C57BL/6 anti-PC antibodies contain molecules of which one-third express the T15 L chain, one-third the M603 L chain, and one-third the M167 L chain. The variations in the frequencies of V_HT15 expression could be explained by assuming that the epitope recognized by the TC54 antibody involves the D segment, and that V_HdT15^a is coexpressed with different D regions than, for instance, V_HT15^b. Although we cannot conclusively exclude this possibility, we must point out that the V_HT15^b so far sequenced use the same D region as their V_HT15^a counterparts (39). An alternative and more likely explanation for our findings can be traced at the level of H-L chain pairing during B cell development. It is now well established that intracellular Ig assembly follows an ordered sequence of events in which functional H chains are expressed before L chains. As the degree of H-L chain interactions must primarily depend on the nucleotide sequence of the respective V_H and V_L genes, it follows that L chains must interact with different strength with the various polymorphic forms of the same V_H gene product. In our particular case, V_HT15^a could, for instance, interact with a larger number of V_k gene products than V_HT15^b. As, apparently, V_k genes segments are expressed at the same frequency, and as accumulation of intracellular H chain appears to be toxic, the end result would necessarily be different frequency of expression of the various allelic forms of the same gene. We recently observed, in full support to this hypothesis, that in normal sera, L chains associated to V_HT15^a are by far more heterogenous than those associated to V_HT15^b. Our frequency analysis revealed that the number of anti-PC precursors was always dominant on the one of V_HT15^+ B cells. This implies that all strains of mice have the potential to use various V_H genes for the production of anti-PC antibodies. At the normal serum level, however, V_HT15 was clearly associated with anti-PC antibodies in BALB/c mice. Indeed, our absorption experiments revealed that the totality of naturally occurring anti-PC antibodies of BALB/c and BALB.B mice was recovered in the purified V_HT15^+ fraction. Surprisingly, the anti-PC activity of C58 and SM mice, which have the same Igh-V region as BALB/c, was not completely dominated by V_HT15^+ molecule. A similar observation was also reported by Lieberman et al. (44) who found that

the T15 idiotype was present on naturally occurring anti-PC antibodies of some but not all strains carrying the Igh-V^a haplotype. Interestingly, naturally occurring anti-PC antibodies of C57BL/6, C.B20, C3H, and SJL mice made no use at all of V_HT15. Upon immunization with PC-LPS, however, C57BL/6 mice produce anti-PC antibodies that react with TC54 (22). This shift in the quality of anti-PC antibodies after immunization may indicate that the determinants presented to the immune system by natural PC antigens are quite different from those recognized upon hyperimmunization. An alternative explanation for this phenomenon could be found on the basis of recent evidence demonstrating a shift in V_H utilization during the secondary response to oxazolone (45). Such a shift has been interpreted to represent a better starting point from which high-affinity antibodies can be produced following few key mutations. Following this observation, we can propose that V_HT15^b, contrary to V_HT15^a, is not the best alternative in terms of PC binding, and that this gene segment is only used after hyperimmunization to provide alternative source for somatic mutations. Such a mechanism would also provide a clear explanation for the evolutionary pressure that maintains in the C57BL/6 genome a V_H gene that is expressed at such a low frequency.

Our data, in any case, establish that V_H polymorphism has profound influences not only on the frequency to which the relevant gene products become available in the preimmune repertoire, but also on the specificity of the antibodies to which this gene product is associated.

In conclusion, we suggest that, through stochastic events, preimmune B cells randomly use V_k genes. Such random expression, however cannot be applied to V_H genes, probably as a consequence of their extensive polymorphism. Following antigen challenge, B cell populations are selected asymetrically and this selection leads to an immune repertoire that no longer reflects the probability of expression of each V_H gene segment.

Summary

Using mAb that selectively recognize the various allelic forms of the V_HT15 and V_k21D-E genes' products, we analyzed the influence of V_H and V_k polymorphism on the probability of expression of these gene segments. Our data show that the frequency to which the V_HT15 gene product becomes available in the preimmune repertoire is strongly influenced by the polymorphism of the relevant structural gene, suggesting therefore that V_H genes cannot be randomly used in the various strains. Contrary to this, the frequency of $V_k21D\text{-}E^+$ clones is similar in all mouse strains tested, and in all cases is higher than the frequency of V_HT15 clones. This observation strongly suggests that V_k genes can be randomly expressed, and/or that their number is lower than that of their V_H counterpart. Finally, analysis of the specificity associated to the expression of the V_HT15 segment revealed that V_H polymorphism strongly influences not only the probability of expression of each V gene, but also the specificity of the antibodies on which these V_H genes are used.

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