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RESEARCH ARTICLE

Influence of Clonal Selection on the Expression of Immunoglobulin Variable Region Genes

Tim Manser, Shu-Ying Huang, Malcolm L. Gefter

Vertebrates can produce a humoral immune response to a large number of different foreign antigens because B lymphocytes are able to synthesize immunoglobulins having many different antigen binding specificities. The diversity in structure of variable (V) regions, the antigen binding domains of immunoglobulins, results in diversity of antigen binding specificity. Each resting B cell in the lymphocyte population expresses immunoglobulin molecules with a single V region structure, and thus a single or limited number of binding specificities, as cell surface receptors. A subset of these cells are stimulated to grow and secrete antibody at the onset of an immune response partly as a result of foreign antigen being bound to surface immunoglobulin (*1, 2*).

Immunoglobulin V domains are formed by the association of heavy (H) and light (L) chain V region polypeptides. Molecular analysis of immunoglobulin genes in humans and mice show that transcriptionally active V genes are constructed in the DNA of the B cell lineage by fusion of gene segments that are separated in germ line DNA (*3, 4*). Segments of V genes are members of heterogeneous multigene families (*5–9*). Heavy chain variable region genes are formed by the fusion of three gene segments, V_H, D, and J_H (*4, 6*), whereas light chain variable region genes are formed from two segments, V_L and J_L (*3, 5*). The V region structural diversity, directly encoded in germ line V gene segment families, is amplified as the result of (i) association of different combinations of segments during V gene formation (combinational diversity), (ii) variation in the joining sites of gene segments (junctional diversity) (*5, 10*), (iii) somatic mutation of assembled V region genes (*11–15*), and (iv) association of different V_H and V_L polypeptides during immunoglobulin assembly.

The genetic potential for V region diversity in mouse and man is therefore now well defined. It is not known, however, how much of this potential diversity is actually expressed as functional diversity, that is, diversity expressed in the V regions of B cell surface receptors that can interact with foreign antigen at the onset of an immune response (such as in the preimmune V region repertoire). Previous serological and antigen binding analyses of antibodies produced by B cells derived from unimmunized mice indicate that the number of different antigen binding specificities in the preimmune V region repertoire is extremely large ($\sim 10^7$) (*16, 17*) and that any single V region structure is likely to be expressed at a very low frequency in this repertoire (*18, 19*). It is generally assumed that combinational, junctional, and possibly mutational processes contribute to the diversity of V region structures in the preimmune repertoire. In fact, little is known concerning the relative contribution of these sources of diversity and whether or not functional restrictions to the random assortment and modification of V gene segments and their polypeptide products exist.

For several years we have used the immune response to para-azophenylarsonate (Ars)–protein conjugates in strain A mice as a model immune response. This response is characterized by the reproducible appearance in the serum of a family of antibodies containing V regions that bear serologically cross-reactive determinants (idiotype) and comprise an average of 50 percent of all hapten binding antibodies (*20*). Molecular characterization of monoclonal Ars-binding antibodies that express these idiotypic determinants (termed Id^{CR}), and the genes that encode them, has revealed that a single V_H gene segment (V_HId^{CR}) participates in encoding all the V_H regions in these molecules (*15*). Amino acid sequences of the V_L regions of such molecules suggest that a single V_L gene segment, in combination with a single J_L segment, encodes these polypeptides (V_LId^{CR}) (*21, 22*). The dominant cross-reactive idiotypic family of antibodies elicited with Ars in strain A mice is therefore analogous to other major idiotypic families elicited in inbred strains by other antigens (*23*). The idiotype-bearing V regions expressed in these families are often encoded by small numbers of related V_H and V_L gene segments in combination with multiple, heterogeneous D and J gene segments. The V_HId^{CR} gene segment is, however, associated with both the J_H2 gene segment and an extremely homogeneous family of D region gene segments in the expressed V_H genes of hybridomas that synthesize Ars-binding Id^{CR}-bearing molecules (*24, 25*). Thus, a very limited amount of combinational diversity is ob-

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Abstract. *The humoral immune response of the mouse to certain antigens is characterized by the dominant expression of a single or limited number of related, immunoglobulin variable region (V) structures by antibody-secreting lymphocytes. Such dominance could be due to preferred expression of these V regions in the B cell population prior to the immune response or could result from the action of selective or regulatory mechanisms during the immune response. Expression of a heavy chain variable region (V_H) gene segment that partially encodes a V region structure that dominates the immune response to para-azophenylarsonate (Ars) in strain A mice was examined in the B cell population of Ars nonimmune mice. This V_H gene segment participates in encoding several hundred thousand different V region structures expressed in this B cell population. The immune system is therefore capable of recurrently selecting a single V region structure from such a repertoire for dominant expression by antibody-secreting lymphocytes during an immune response.*

served among Ars-binding antibodies that express Id^{CR} determinants.

Extreme homogeneity of V region structure among most of the antibodies elicited with any antigen seems surprising, in view of the great potential for V region diversity directly encoded in V gene segment families and the diversity that could be created by the unrestricted association of these elements and their V_H and V_L polypeptide products. Indeed, Ars-binding immunoglobulins containing various V regions encoded by V_H and V_L genes unrelated to V_HId^{CR} and V_LId^{CR} are expressed in this immune response (*26, 27*). In addition, we have recently found that hybridomas that synthesize Ars-binding antibodies (anti-Ars) encoded by V_HId^{CR}, V_LId^{CR}, and a diverse group of D and J_H gene segments can be isolated, at a low frequency, from strain A mice undergoing an Ars immune response (*28*). Dominance of this extremely homogeneous Id^{CR} population of V region structures is therefore enigmatic.

In order to address the question of why the Id^{CR} combination of gene segments reproducibly dominates V gene expression during the anti-Ars response of strain A, we examined the V region repertoire of these mice before immunization. If a disproportionately large number of resting B cell clones express V region structures encoded by the Id^{CR} gene segment combination, these structures would tend to dominate the anti-Ars response. A recent report suggests that clonal dominance prior to the immune response may be responsible, in part, for the serum dominance of the T15 idiotypic family of antibodies elicited in BALB/c mice with phosphorylcholine (*29*). Conversely, if the V region repertoire is formed in a stochastic manner through the random fusion of gene segments and association of V_H and V_L polypeptides, the V region structure encoded by the Id^{CR} combination of gene segments should be found at a frequency determined entirely by the total number of V region gene segments in the mouse genome. Should this be true it would indicate that the Id^{CR} combination of gene segments is "singled out" of a large population of gene segment combinations that are equally represented in the preimmune repertoire.

We have developed a hybridoma screening technique (lysate hybridization) that allows cell lines to be selected from a large population simply on the basis of homology of cellular messenger RNA (mRNA) with a DNA probe (*30*). We described the partial characterization of several B cell hybridoma cell lines that were produced from unimmunized A/J mice and identified with this technique and that express the V_HId^{CR} gene segment (*30*). Extension of this analysis has allowed us to determine the frequency of V_HId^{CR} expression in the B cell population prior to the Ars immune response and to evaluate the combinational, junctional, and mutational diversity present in the context of this V_H region gene segment.

B cell hybridomas derived from mice not immune to Ars. Polyclonal B cell mitogens were used to stimulate resting B cells from the spleens of Ars nonimmune A/J mice to grow and secrete antibody. Hybridomas were constructed from the stimulated B cells of individual mice by fusion with the transformed lymphoid cell line SP2/O. Mitogenic stimulation was required because resting B cells form hybridomas very inefficiently. The hybrid cell populations were screened by means of the lysate hybridization technique with a V_HId^{CR} specific DNA probe, which was derived from the region encoding amino acids 15 to 58 of the germ line V_HId^{CR} gene segment (*31*). Initial experiments had shown that some cross-hybridization to mRNA's containing related V_H gene sequences occurs under our hybridization conditions. However, hybridoma colonies that give rise to strong hybridization signals are the only colonies that synthesize antibodies that are recognized by AD8, a rat monoclonal antibody (*32*) that binds an idiotypic determinant encoded in V_HId^{CR}, whereas weaker hybridizing colonies do not. Previous experiments also demonstrated that varying amounts of hybridization signal were due to the degree of homology of the expressed V regions with the V_HId^{CR} probe and not to varying amounts of V_H region mRNA per cell. For these reasons we think that all strong V_HId^{CR} hybridizing, AD8-positive hybridomas express the V_HId^{CR} gene segment.

Using the V_HId^{CR} probe we screened hybridomas derived from A/J splenic B cells stimulated with bacterial lipopolysaccharide (LPS) (Table 1). The frequency of strongly hybridizing colonies that synthesize antibodies recognized by AD8 is approximately 1 in 400 colonies;

Table 1. Splenic B cells from A/J mice (6 to 8 weeks old) were mitogenically stimulated with either bacterial lipopolysaccharide (75 μg/ml) or affinity-purified goat antibody to mouse immunoglobulin μ chain (75 μg/ml), fused to SP2/O; the hybridomas were selected and screened by lysate hybridization with the use of a ^{32}P-labeled V_HId^{CR} DNA probe as described (*30*). Positive hybridomas were isolated and culture supernatants were assayed for AD8 reactive immunoglobulin by means of a solid-phase competition radioimmunoassay (*30, 57*).

Screen number	Mitogen	Hybridomas screened (No.)	V_HId^{CR} hyb^+, $AD8^+$	Frequency	Hybridomas obtained
3	LPS	900	2	1/450	hVH65-5
4	LPS	400	1	1/400	hVH65-8
					hVH65-2
					hVH65-7
8A	LPS	1200	3	1/400	hVH65-17
					hVH65-20
9	LPS	1350	3	1/450	hVH65-21
					hVH65-22
11	LPS	1350	5	1/270	hVH65-25
13-8	Gαμ	250	1	1/250	hVH65-αμ1
19	Gαμ	3400	12	1/285	hVH65-αμ2
					hVH65-αμ5

nearly all of these colonies synthesize immunoglobulin M (IgM) heavy chain mRNA, as assayed by lysate hybridization with an IgM (C_μ) constant region gene probe. Although LPS is known to stimulate approximately 30 percent of all A/J splenic B cells (*33*), we considered the possibility that these LPS reactive cells did not express the entire functional V region preimmune repertoire. For this reason another B cell mitogen, goat antibody to mouse immunoglobulin μ chain (Gαμ), was also used. Recent evidence has suggested that at least two functionally distinct cell subsets exist in the B cell population of the mouse (*34*), and it has been suggested that certain V gene segment combinations may be expressed predominantly in one subset or the other (*35*). B cells that are members of one of these subsets are refractory to mitogenic stimulation with Gαμ (*36*) but are responsive to LPS. The hybridomas we have examined should therefore be derived from both B cell subsets. Since LPS stimulates approximately 30 percent of all A/J splenic B cells and Gαμ stimulates approximately 50 percent (*36*), the entire functional V region preimmune repertoire should be sampled with the use of these two reagents. We found that hybridomas obtained from Gαμ-stimulated splenic B cells express mRNA highly homologous to V_HId^{CR} and AD8-reactive antibody at a frequency of approximately 1 in 300 (Table 1), in agreement with the results obtained with LPS. It has been estimated that there are several hundred V_H region gene segments per haploid mouse genome (*8*). Thus the V_HId^{CR} gene segment is apparently expressed at a frequency expected for stochastic expression of different V_H gene segments. A more detailed examination of the frequency of expression of the V_HId^{CR} gene segment, and of other V_H gene segments, in libraries of hybridomas derived from the splenic B cells is in progress (*37*).

Molecular analysis of V region genes expressed in "preimmune hybridomas." To unambiguously determine whether our isolated hybridomas that produce mRNA highly homologous to the V_HId^{CR} probe and antibodies that react with AD8 express the V_HId^{CR} gene segment, we subjected the variable region of the H chain mRNA synthesized by such cell lines to direct mRNA sequence analysis. The 3′ half of the V_H regions expressed in eight of these hybridomas (hVH65-5 through hVH65-αμ2), obtained from seven different mice, are shown in Fig. 1. At all nucleotide positions that could be unambiguously determined, the sequences of these V_H regions agree with that of V_HId^{CR}. Nucleotide changes from the V_HId^{CR} sequence in two V_H genes present in the A/J genome that are highly homologous to the V_HId^{CR} gene segment (*15*) are shown above the V_HId^{CR} sequence. Since the V_HId^{CR} probe contains sequences from the 5′ half of V_HId^{CR}, and hybridizes at high stringency to the H chain mRNA synthesized by these cell lines, the unsequenced portions of the V_H regions expressed in these preimmune hybridomas are highly homologous to V_HId^{CR} as well. Whole genomic Southern blotting analysis (*38*) with the V_HId^{CR} probe has also demonstrated that DNA's isolated from these cell lines give rise to bands of electrophoretic mobilities diagnostic for the productive rearrangement of the V_HId^{CR} gene segment (*15, 39*).

"Weakly hybridizing" hybridomas that produced AD8-negative antibodies could express the V_HId^{CR} gene segment as well, possibly in a form greatly altered by somatic mutation. However, nucleotide sequences of the 3′ half of the V_H regions expressed in two such hybridomas (hVH65-2 and hVH65-7), isolated from a single A/J mouse, are more homologous to one another than they are to V_HId^{CR} (Fig. 1). These two V_H genes could not have been derived from the same V_H gene precursor via somatic mutation since they contain different D and J_H gene segments (data not shown), and were most probably not derived from the germ-line V_HId^{CR} gene segment since eight identical somatic mutation events would have had to occur in V_HId^{CR} to generate these highly related genes. These V_H regions must then utilize one or two other germ-line V_H gene segments.

In contrast to the homogeneous combination of V_HId^{CR}, D and J_H2 gene segments expressed in hybridomas that produce Id^{CR}-bearing, Ars-binding antibodies, preimmune hybridomas express V_HId^{CR} in the context of all four J_H gene segments and many D segments that differ in length and sequence. The sequences of the various D regions expressed in these hybridomas and the consensus D sequence found in the Id^{CR} combinations of gene segments are shown in Fig. 2. Expressed J_H gene segments are also indicated. The "core nucleotides" of most of these D regions

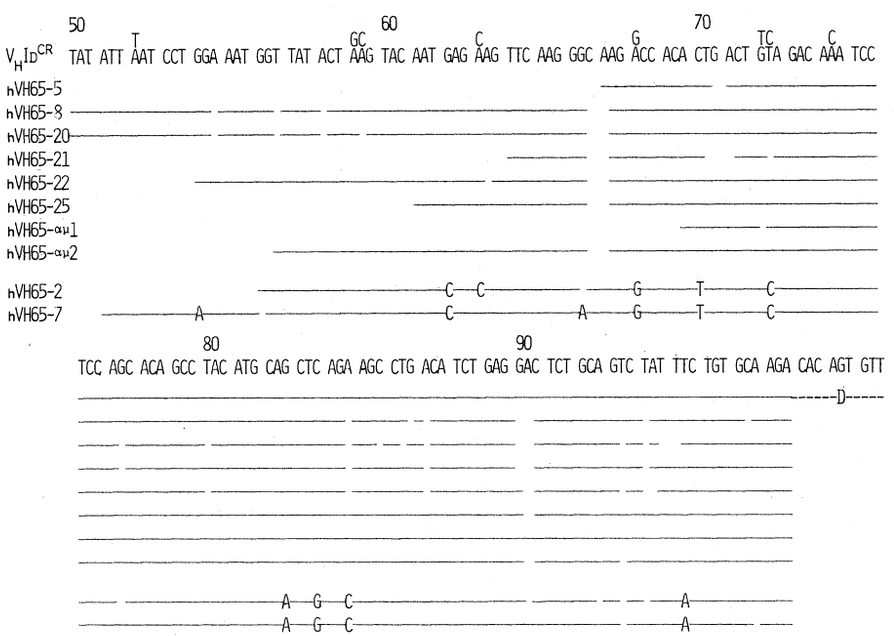


Fig. 1. Nucleotide sequences of the expressed V_HId^{CR} region of preimmune hybridomas. Nucleotide sequences of the 3′ halves of the expressed V_H regions of preimmune hybridomas are compared to the sequence of the germ-line V_HId^{CR} gene segment (*15*). The hVH65 sequences were obtained by direct sequencing of H chain mRNA with the use of an oligonucleotide primer and reverse transcriptase (*53, 54*). Solid lines denote sequence identity. Nucleotide differences are shown explicitly. Gaps represent nucleotides that could not be unambiguously identified. Nucleotide differences in two V_H gene segments present in the A/J genome that are highly related to V_HId^{CR} (*15*) are shown above the V_HId^{CR} sequence. Amino acid codons are numbered sequentially starting with the mature amino terminus. No homology to the V_HId^{CR} gene segment is present following codon 98 (AGA) in these expressed V_HId^{CR} regions. Sequences following this codon presumably represent the D region (dashed lines) of these expressed V_H regions. Hybridomas hVH65-5 through hVH65-αμ2 synthesize mRNA highly homologous to the V_HId^{CR} probe and antibodies that react with AD8. Hybridomas hVH65-2 and hVH65-7 synthesize mRNA less homologous to the V_HId^{CR} probe and antibodies that do not react with AD8.

appear to be derived from members of the germ-line D gene segment family termed the "SP2 family" (*11*). As is often found at V_H-D and D-J_H junctions (*11*), nucleotides are present that cannot be accounted for by the coding or flanking sequences of the putative constituent germ-line V gene segments of these V_H genes (represented by small letters in Fig. 2). In Fig. 2 the D region nucleotides that appear to have been derived from germ-line D gene segments (characterized in the BALB/c genome) are aligned to maximize homology with the SP2 D "core" sequence and are boxed. Varying numbers of nucleotides are contributed to each D by the germ-line segments. These core D regions are all highly homologous, as is observed among the germ-line D gene segment family members. Interestingly, the germ-line D-derived nucleotides are always utilized in the same relative reading frame within the expressed D regions. This indicates that, despite their size, D region core sequences behave as small polypeptide encoding genes, not simply as sources of nucleotide stretches. The core sequences of three of these expressed D regions differ from the highly conserved SP2 D family core sequence by a single G to A transition (marked with an overlying asterisk). This may be a sequence polymorphism between the SP2 D families of A/J and BALB/c mice or may represent a previously uncharacterized D gene segment.

In the different $V_H Id^{CR}$-containing V_H regions that utilize the same J_H segment this segment has been joined to the D gene segment at different locations within germ-line J_H sequences (Fig. 3). The site of D-J_H joining varies over several nucleotides for different joints involving the same J_H, producing a corresponding variation in the amount of 5′ J_H information donated to the functional V_H gene. Such junctional diversity has been observed among a variety of expressed V_H and V_L genes (*40*). Unexpectedly, the $V_H Id^{CR}$-D junction sequences show no evidence of this "joining imprecision." In all cases, the V_H-D joint has occurred at the same location in the $V_H Id^{CR}$ gene segment. In other V_H genes that utilize the same V_H gene segment V_H joining site variation has been observed (*41*).

The light chain V regions expressed in two hybridomas were also analyzed by nucleotide sequencing. The 3′ half of these V_L regions are shown in Fig. 4 in nucleotide and translated form compared to the consensus $V_L Id^{CR}$ amino acid sequence. The codons for highly conserved V_κ region amino acids (*42*) are underlined. These two hybridomas express different V_L genes that are unrelated to $V_L Id^{CR}$. To determine whether other preimmune hybridomas were also expressing different V_L genes, genomic Southern blots of Bam HI–digested DNA's from these hybridomas were hybridized with a probe containing the BALB/c germ line J_κ gene segment locus (all of these hybridomas express light chains of the kappa isotype) (Fig. 5). This probe hybridizes to a 13.5-kb restriction fragment containing the unrearranged J_κ and C_κ genes, to a 7.2-kb restriction fragment containing the SP2/O-derived rearranged V_L-J_κ-C_κ gene, and to a fragment of varying size containing the A/J-derived V_L-J_κ-C_κ rearranged gene. The size of this variable-sized fragment is determined by two factors—the V_L gene segment that is productively rearranged and the J_κ element to which it fuses. The variation in sizes of the A/J-derived V_L-J_κ-C_κ restriction fragments (Fig. 5) demonstrates that either the preimmune hybridomas express different V_L gene segments or express the same V_L segment in combination with many J_κ gene segments. Since the distance between J_κ segments is known (*5, 6*), these two possibilities can be distinguished for any two V_L-J_κ-C_κ fragments by careful measurement of their molecular weights. It appears that at least four different V_L gene segments are productively rearranged among these seven hybridomas and that all the rearranged V_L genes utilize different V_L-J_κ combinations.

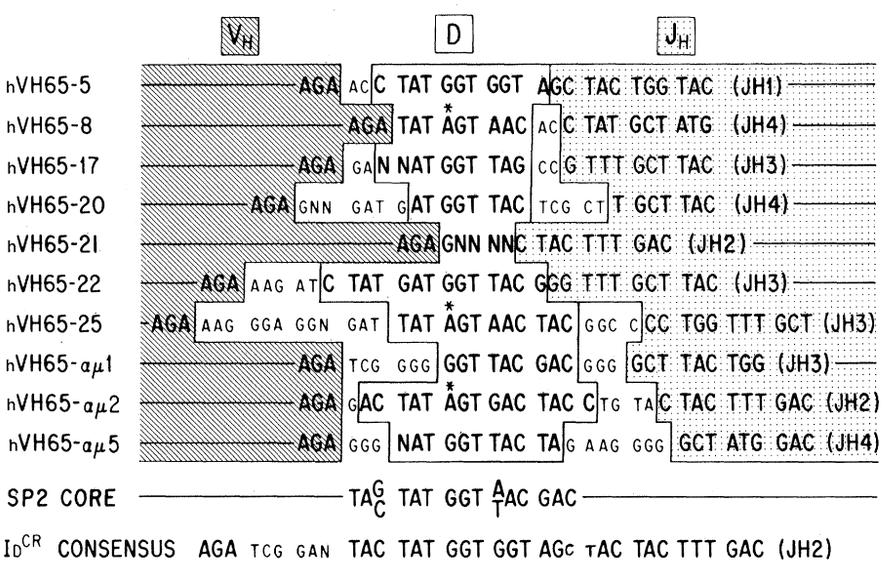


Fig. 2. Nucleotide sequences of the V_H-D-J_H region in the expressed V_H region genes of preimmune hybridomas that utilize the $V_H Id^{CR}$ gene segment. The nucleotide sequences are compared, beginning with the 3′ terminal AGA codon derived from the $V_H Id^{CR}$ gene segment, to the "core" sequence of the germ-line D gene segment family of BALB/c termed SP2 (*9*) and the consensus D sequence found in the expressed V_H genes of hybridomas that produce Id^{CR}-bearing, Ars-binding antibodies. Sequences have been aligned to produce the maximum homology with the SP2 core sequence. Nucleotides that appear to be derived from germ-line V gene segments are boxed. Nucleotides that cannot be accounted for by the sequences of the putative germ-line gene segments that were fused to form these functional V_H genes are shown in small lettering. Nucleotides that could not be unambiguously identified are indicated by "N." Three nucleotides that may represent a G to A polymorphism between some BALB/c and A/J SP2 D gene segments are indicated with an overlying asterisk.

Preimmune V Region Repertoire Appears to Be Formed Stochastically

We have found that the $V_H Id^{CR}$ gene segment is expressed in the B cell population in the context of an extremely diverse group of additional V gene segments. This suggests that expression of the homogeneous family of $V_H Id^{CR}$ utilizing V region structures during the immune response of A/J mice to Ars does not result as a consequence of events that precede the Ars response. Among the ten preimmune hybridomas that express $V_H Id^{CR}$ we have examined, all express different D regions. All four J_H gene segments are utilized, and at least four different V_L gene segments in six different V_L-J_L combinations are expressed. Combined with the frequency of expression of $V_H Id^{CR}$ among preimmune hybridomas (~1/400), these findings indicate that a maximum of one in every 10^5 (400 × 10 D's × 4 J_H's × 6 V_L-J_L's) B cell in the Ars nonimmune mouse could express a V gene segment combination similar to those that encode Ars-binding, Id^{CR}-bearing V regions. In fact, the frequency of expression of such

a combination is probably much lower since our results indicate that there is little, if any, restriction on the mechanisms that generate combinational and junctional diversity in the context of V_HId^{CR}. Preliminary analysis of the expression of another V_H gene segment in the B cell population suggests that random V gene segment assortment in the context of single V_H gene segments is a general phenomenon (*37, 43*). If we assume that this is the case, a minimum (ignoring junctional diversity) of 10^5 (300 V_L's × 4 J_L's × 4 J_H's × 20 D's) different V gene segment combinations involving V_HId^{CR} are expressed in the preimmune repertoire—a value that is based on current estimates for the size of V gene segment families in the mouse genome (*40, 44*)—and 1 out of 4×10^7 ($10^5 \times 400$) B cells expresses the Id^{CR} gene segment combination. Nevertheless, this combination is reproducibly expressed during the immune response to Ars.

The reason for the dominance of Id^{CR} antibodies in the immune response of A/J mice to Ars remains unclear. It appears that this dominance is created by selective forces that act on B cells during, not prior to, this immune response. These forces must act to select V regions encoded by the Id^{CR} gene segment combination from a diverse population of other V region structures encoded by other combinations that include the V_HId^{CR} gene segment, some of which bind Ars. What properties of Id^{CR} V regions might be recognized by such selective forces? The clonal selection theory, first proposed by Burnet (*45*), states that antigen binding to the surface immunoglobulin of a B cell clone selects that clone for participation in the immune response and suggests that the affinity of surface immunoglobulin for antigen may influence this process (*46*). The network hypothesis, proposed by Jerne (*47*), states that participation of B cell clones in an immune response does not depend entirely on the antigen specificity of the V region these clones express but on idiotypic determinants that are part of a large idiotype–anti-idiotype regulatory network as well. Evaluation of which, if either, of these two hypotheses could best account for the dominance of Id^{CR} antibodies in the A/J immune response to Ars is beyond the scope of this discussion (*48, 49*).

No evidence for alteration of the germ line V_HId^{CR} sequence by somatic point mutation in the V regions expressed by the hybridomas we have isolated has been obtained. V_HId^{CR} gene segments expressed in hybridomas obtained from mice undergoing an immune response to Ars contain numerous mutational alterations (*15, 28*). This suggests that the process of somatic mutation may only occur during the immune response to Ars. These data agree with the findings of Gearhart *et al.* (*41*) and Bothwell *et al.* (*50*), who observed that V regions derived from single germ-line V_H gene segments were unmutated when ex-

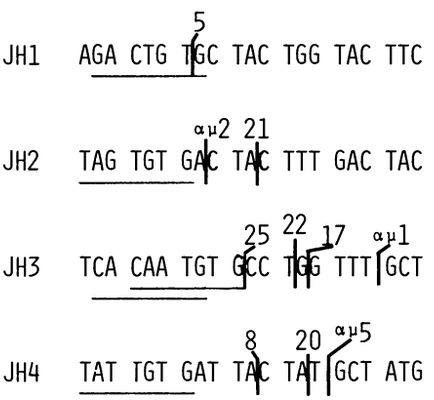


Fig. 3. The J_H joining sites used during the formation of the V_H regions expressed in preimmune hybridomas. The germ-line sequences of the 5′ coding and flanking sequences of the four BALB/c J_H gene segments are shown (*4, 6*). The apparent location of the "J_H ends," which were ligated to D sequences during the formation of the expressed V_H genes in the hVH65 preimmune hybridomas indicated, are represented by vertical bars. "Signal" heptamer sequences (*40*) in the 5′ flanking sequences of the J_H elements are underlined.

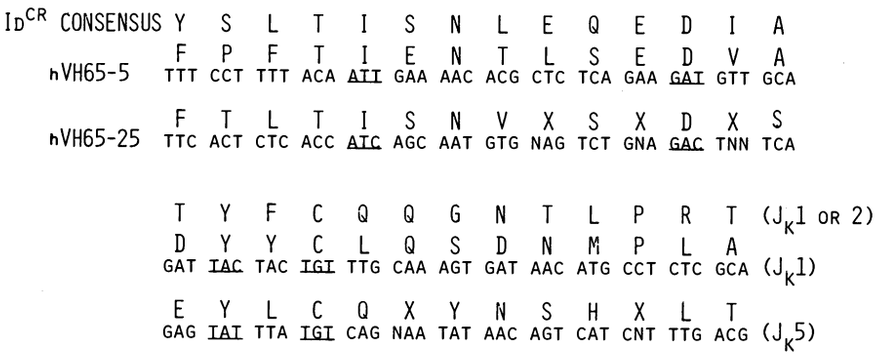
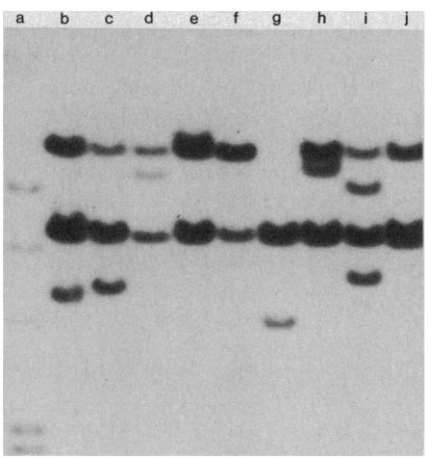


Fig. 4. The V_L gene segment coexpressed with V_HId^{CR} in two preimmune hybridomas. The 3′ regions of the V_κ gene segments expressed in the preimmune hybridomas hVH65-5 and hVH65-25 are shown in nucleotide and translated form as compared to the consensus sequence of V_LId^{CR} light chain V regions in this area (*21, 22*). Codons for conserved amino acids, found in most V_κ regions at specific locations (*42*), are underlined. The J_κ gene segments used in conjunction with these V_κ's are indicated. Single letter abbreviations for amino acid residues are A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; L, leuceine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; Y, tyrosine; and X, ambiguous.

Fig. 5. Southern blot of preimmune hybridoma DNA's with the use of a J_κ probe. Hybridoma DNA's were digested with Bam HI and subjected to electrophoresis on a 0.5 percent agarose gel. The DNA was blotted to nitrocellulose and the filter was hybridized with a ^{32}P-labeled restriction fragment containing the BALB/c J_κ locus (*55*) as described (*56*). The lanes are, from left to right, (a) λ DNA digested with Hind III and ^{32}P end-labeled with polynucleotide kinase; (b) hVH65-5; (c) hVH65-8; (d) hVH65-17; (e) hVH65-20; (f) hVH65-21; (g) hVH65-22; (h) hVH65-25; (i) 36-65 (a hybridoma that expresses V_HId^{CR} and V_LId^{CR}); and (j) SP2/O (the fusion partner used to construct all these hybridomas). The sizes of all the rearranged bands generated from hVH65 DNA's were measured (hVH65-21 either lost its A/J rearranged C_κ locus or gives rise to a band indistinguishable in molecular weight from the 13.5 or 7.2 kb bands). These values indicate that at least four V_κ gene segments and six V_κ-J_κ combinations are used among these six preimmune hybridomas (see text).



pressed in IgM's but often contained mutational alterations when expressed in IgG's. IgM synthesis ontogenically precedes IgG synthesis during the course of an immune response (*51*). IgG-producing B cells are derived directly from IgM-producing cells via heavy chain constant region gene "class switching" (*52*). All of the preimmune hybridomas we have isolated express IgM and therefore our data do not address the question of whether somatic mutation events are associated with class switching.

The available data indicate that the preimmune V region repertoire is created using V gene segment information in germ-line form. The size of this repertoire is ultimately limited by the number of B lymphocytes in the animal. In the mouse, the number of possible gene segment combinations approaches the total number of B cells. Germ-line V gene segment sequences have presumably evolved under the influence of at least two selective forces; the need to maintain V region structural integrity to ensure that functional combining sites are formed, and the need to maximize the diversity of binding specificity expressed in the limited number of B cells in the immune system. Somatic mutation seems to occur at random locations in V genes (*40*) and thus may have a significant probability of destroying V gene function rather than creating V genes that encode new binding specificities not directly encoded in the germ-line genome. Somatic mutation may therefore not function during the formation of the preimmune repertoire to ensure that the greatest possible number of different functional combining sites are expressed prior to the appearance of foreign antigen.

We have demonstrated that extensive combinational and junctional diversity is expressed in the context of a single V_H region gene segment in the B cell population of the mouse prior to an immune response. The considerable amount of diversity present in the context of the V_HId^{CR} gene segment suggests that there is little restraint on the assembly of different germ-line V gene segments during B cell differentiation. In addition, it appears that many different combinations of V_HId^{CR} and V_L polypeptides can associate to form functional V region domains. Presumably these different combinations of gene segments and polypeptides give rise to antibodies with different antigen binding specificities (all of the preimmune hybridomas we have examined produce antibodies that have no detectable affinity for Ars). Our data indicate that combinational and junctional processes contribute most of the diversity to the preimmune V region repertoire. A very limited amount of combinational and junctional diversity is found among antibodies that are partially encoded by the V_HId^{CR} gene segment and are elicited by Ars immunization. Thus strong selective forces act on the B cell population during the Ars immune response, resulting in dominant expression of a single combination of V gene segments. The nature of these selective forces remains a subject for further investigation.

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