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# Organization of the murine immunoglobulin $V_H$ complex in the inbred strains

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**Deletion mapping analyses have been employed to order the heavy chain variable region ($V_H$) gene familes in three inbred murine strains. These nine $V_H$ gene families have been positioned with respect to the J558 and 3660 $V_H$ families in A/J ($Igh^e$) as follows: 3609-J558-(J606,VGAM3-8,S107)-3660-(X24,Q52,7183)-$D_H$. Maps generated with respect to J558 in the BALB/c ($Igh^a$) and C57BL/6 ($Igh^b$) strains are consistent with these results. The organization of the $V_H$ complex produced by deletion mapping is quite different from the accepted map generated by other methods, particularly in that J558 is more $D_H$ distal and 3660 is more $D_H$ proximal than previously thought. The order presented here is compatible with $V_H$ rearrangement frequencies suggesting preferential utilization of $D_H$-proximal $V_H$ gene segments. Our data also indicate that interspersion of some $V_H$ family members may be a common feature of the murine $V_H$ complex since the 3609 $V_H$ family is interdigitated in the three strains and a Q52 $V_H$ gene segment is interspersed in C57BL/6.**

*Key words:* immunoglobulins/$V_H$ gene families/murine/deletion mapping/gene order

## Introduction

Expression of immunoglobulin heavy chains depends upon the rearrangement and juxtaposition of three different germ-line encoded elements. Variable ($V_H$), diversity ($D_H$) and joining ($J_H$) gene segments recombine to form a complete, functional variable region (VDJ) (reviewed in Alt *et al.*, 1986). Murine $V_H$ gene segments have been classified into nine $V_H$ gene families that appear to be clustered in the $V_H$ complex (Kemp *et al.*, 1981; Crews *et al.*, 1981; Givol *et al.*, 1981; Rechavi *et al.*, 1982; Yancopoulos *et al.*, 1984; Brodeur and Riblet, 1984; Brodeur *et al.*, 1984; Dildrop, 1984; Winter *et al.*, 1985; Riblet *et al.*, 1987). These $V_H$ gene families are named on the basis of cross-hybridization to a specific, cloned $V_H$ probe from a particular B cell line (Brodeur and Riblet, 1984); $V_H$ gene segments from different $V_H$ gene families do not cross-hybridize.

The relative positions of $V_H$ gene families with respect to each other were initially proposed on the basis of the heritable patterns of defined idiotypic specificities utilizing several inbred and recombinant inbred strains within a limited number of constant region (IgC) allotypes (Weigert and Potter, 1977; Riblet, 1977). Southern filter hybridization analyses of inbred and recombinant inbred strains have produced a generally accepted map order of eight of these $V_H$ gene families within the murine $V_H$ complex: (3609, J606, 3660, X24)-J558-S107-Q52-7183-$D_H$ (Brodeur *et al.*, 1984; Makela *et al.*, 1984; Riblet *et al.*, 1987). The 3609, J606, 3660 and X24 $V_H$ families have not been mapped relative to each other (Riblet *et al.*, 1987). The VGAM3-8 $V_H$ family is as yet unmapped.

The organization of the $V_H$ families has important implications with respect to understanding the regulation, expression and selection of the expressed $V_H$ repertoire from the germ-line encoded array. Although the mechanisms by which this selection takes place are not fully understood, recent evidence suggests that the frequency of $V_H$ segment utilization reflects its proximity to the rearranged $D_H$-$J_H$ (Yancopoulos *et al.*, 1984; Perlmutter *et al.*, 1985; Wu and Paige, 1986). However, several inconsistencies exist with respect to the $V_H$ family usage frequencies and the accepted $V_H$ map (Riley *et al.*, 1986; Wu and Paige, 1986).

In this study, deletion mapping of B cell lines that contain a single VDJ rearrangement was used to determine the relative order of the nine $V_H$ families in A/J, BALB/c and C57BL/6 inbred strains of mice. The results of these studies are inconsistent with the accepted $V_H$ map but consistent with the proximal usage model of $V_H$ gene families in $V_H$-$D_HJ_H$ rearrangements.

## Results

### *The A/J, BALB/c and C57BL/6 cell lines used in this study contain a single, productive VDJ that rearranged by deletion*

The utilization of deletion mapping to order $V_H$ genes rests on the premise that rearrangement of variable region elements results in excision and loss of intervening DNA (Cory and Adams, 1980; Sakano *et al.*, 1980 reviewed in Alt *et al.*, 1986). A requirement for this procedure is that the $V_H$ complex be represented as a single copy since Southern filter hybridization lacks the sensitivity to consistently distinguish between one or two copies. Equally crucial for deletion mapping is to rule out other recombinational mechanisms or consequences such as inversions, unequal sister chromatid exchange or possible re-integration of excised DNA within the genome (reviewed in Alt *et al.*, 1986).

These criteria were applied to the A/J hybridoma 91A3 which produces a strain-specific anti-arsonate antibody whose amino acid sequence places it in the J558 $V_H$ family (Slaughter and Capra, 1983). When 91A3 DNA was digested with three different restriction endonucleases and hybridized with a $J_H3$-$J_H4$ probe, a single hybridizing fragment was observed in each case, showing that it contains only the productively rearranged allele (Figure 1). Further analysis of this DNA using 5′ and 3′ flanking fragments of the $D_H$ gene segment utilized by these Ars-A hybridomas (DFL16.1$^e$) (Landolfi *et al.*, 1986) and a 5′ flanking fragment of $J_H1$ (91A3 uses $J_H2$) (Slaughter and Capra, 1983) confirmed that the intervening DNA between $D_H$ and $J_H$, and $V_H$ and $D_H$ had been excised and lost from the genome as a consequence of the rearrangement process (Figure 1). This established that 91A3 DNA was suitable for deletion mapping purposes. Similar experiments were performed on the DNA of 94B10, an A/J ($Igh^e$) hybridoma that utilizes a 3660 $V_H$ gene and probably DFL16.1$^e$ (Estess *et al.*, 1979; Milner and Capra, 1984), S43.10 ($Igh^b$) a C57BL/6-derived hybridoma that expresses the $NP^b$ idiotype and uses the J558 186-2 $V_H$ gene and

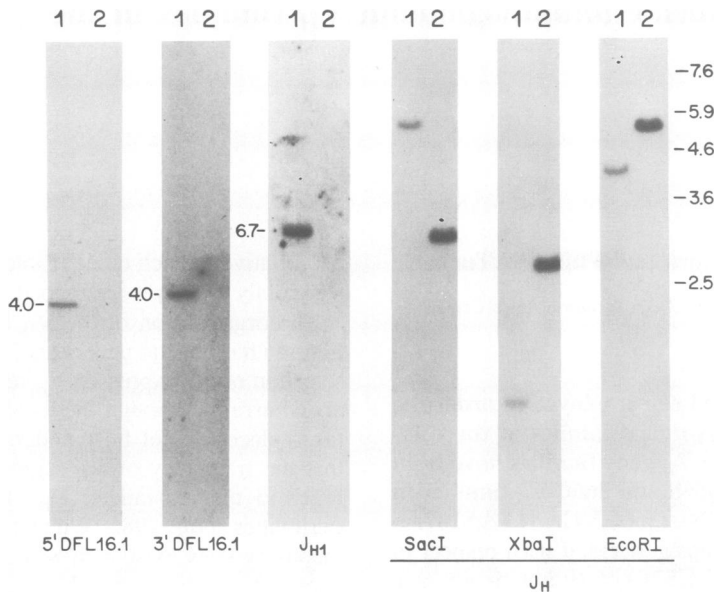


**Fig. 1.** Southern filter hybridization of 91A3 genomic DNA showing that VDJ rearrangement occurred by deletion and that 91A3 is haploid for the $V_H$ complex. The three panels to the left are *Eco*RI digests of A/J liver DNA (**lane 1**) and 91A3 DNA (**lane 2**) probed with 5′ and 3′ flanking regions of DFL16.1[e], and $J_H1$ demonstrating that deletion of intervening DNA between $V_H$, $D_H$ and $J_H$ has taken place in the 91A3 hybridoma. The three panels to the right demonstrate that 91A3 contains a single VDJ rearrangement.

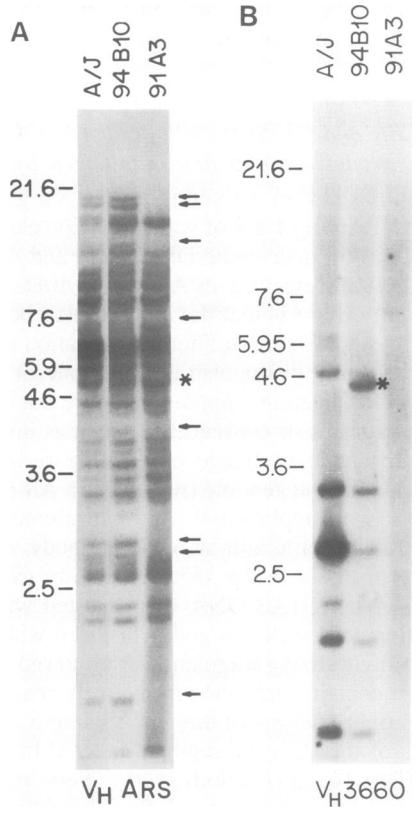


**Fig. 2.** The J558 $V_H$ family is positioned 5′ of the 3660 $V_H$ family in A/J mice. Deletion mapping was carried out on *Eco*RI-digested DNA of A/J liver and hybridomas 91A3 and 94B10 (which utilize a J558 and a 3660 $V_H$ gene segment respectively). Asterisks denote the rearranged $V_H$ genes in each $V_H$ family. Probes are derived from (**A**) 93G7, which recognizes the J558 $V_H$ family and (**B**) M460, which hybridizes to the 3660 $V_H$ family.

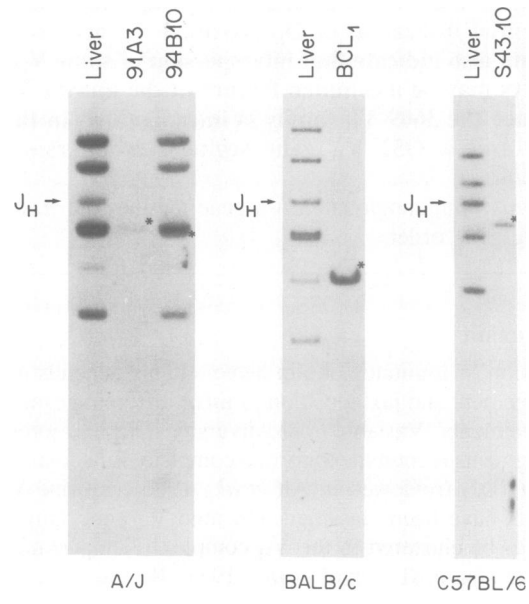


**Fig. 3.** Mapping the VGAM3-8 $V_H$ family in A/J, BALB/c and C57BL/6. A/J liver, 91A3, 94B10, BALB/c liver, $BCL_1$, C57BL/6 liver and S43.10 DNAs were digested with *Eco*RI and probed with the rearranged VGAM3-8 fragment. Since this fragment contains $J_H4$ and sequences 3′ of $J_H4$, it also hybridizes to the rearranged $V_H$ genes in each of the B cell lines (asterisks). '$J_H$' indicates the 6.4-kbp *Eco*RI fragment that contains the $J_H$ gene segments in unrearranged configuration.

DQ52 (Reth *et al.*, 1978; Bothwell *et al.*, 1981), 287.6, a (CBA × C57BL/6) F1 hybrid that utilizes the 186-2 $V_H$ gene and DFL16.1 (Siekivitz *et al.*, submitted), BCL1, a BALB/c B cell lymphoma that uses a J558 $V_H$ gene of undetermined specificity and Dsp2.5 (Knapp *et al.*, 1982) and 5G8, a BALB/c anti-*Haemophilus influenzae* type B lipooligosaccharide hybridoma (Gulig *et al.*, 1987) that uses a J558 $V_H$ gene (data not shown). From these experiments we concluded that each of these six B cell lines contained a single VDJ rearrangement and had lost the intervening DNA between $V_H$, $D_H$ and $J_H$. Karyotypic analyses to determine whether the hybridomas were haploid for the Igh-bearing chromosome 12 or whether they carried two or more identically rearranged VDJ copies were not

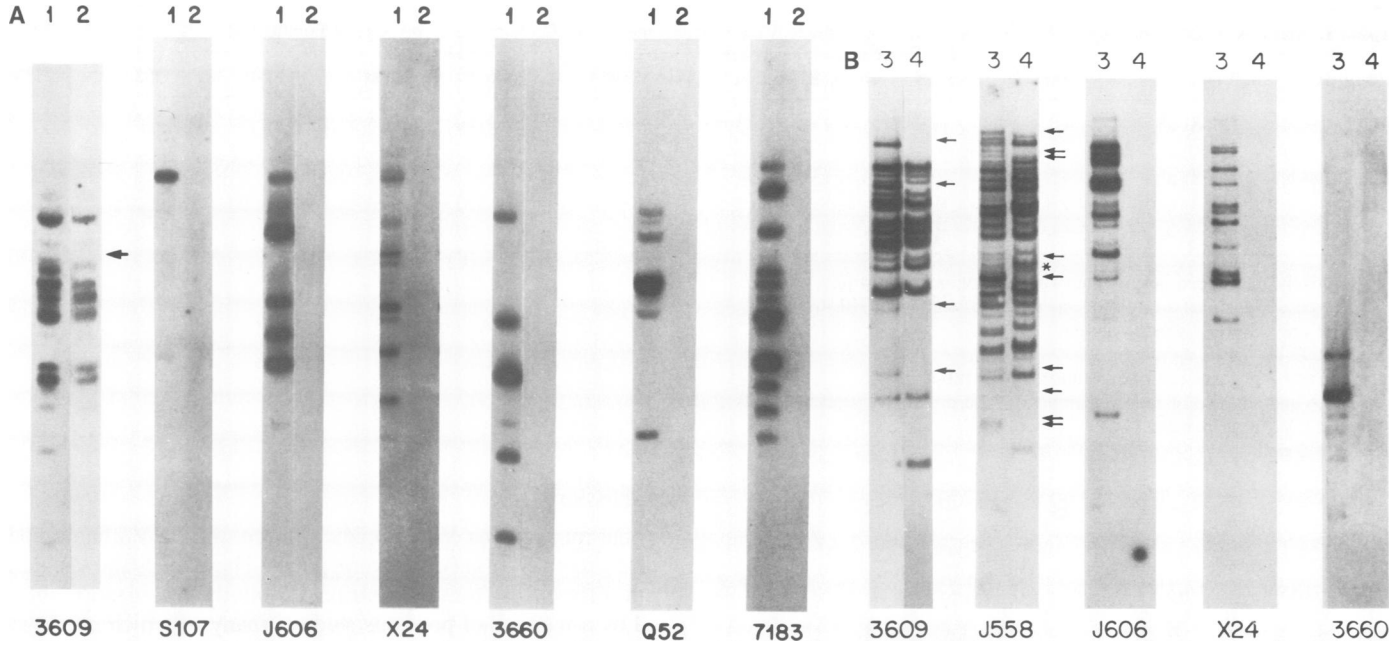


**Fig. 4.** The J558 family is situated 5′ of seven other $V_H$ gene families in the A/J and BALB/c strains. (**A**) Lanes 1 and 2 contain A/J liver and 91A3 DNA respectively, digested with *Eco*RI and probed with the indicated $V_H$ family specific fragment. Arrow indicates missing 5.9-kb fragment hybridizing to a 3609 $V_H$ gene segment. (**B**) *Eco*RI-digested BALB/c liver (**lane 3**) and $BCL_1$ (**lane 4**) DNA were probed with the indicated $V_H$ family fragment. Under conditions of moderate hybridization stringency, the X24 $V_H$ probe cross-hybridizes to several $V_H$ subgroup III gene segments (Hartman and Rudikoff, 1984). Asterisks indicate the rearranged 91A3, $BCL_1$ and S43.10 J558 $V_H$ genes; arrows indicate missing 3609-specific fragments.

performed. However, such an analysis of $BCL_1$ together with extensive restriction mapping analyses confirmed that it carried two apparently duplicated copies of chromosome 12 that contain identical VDJ rearrangements (Chen *et al.*, 1986).

*Deletion mapping of the $V_H$ families in the three inbred strains*

*Eco*RI-digested DNA of two A/J hybridomas (91A3 and 94B10) was probed with a J558 $V_H$ fragment (encompassing amino acid positions −4 to 92) that was derived from the cDNA clone 93G7 (Sims *et al.*, 1982). Figure 2A shows that 91A3 retains most of the J558 $V_H$ gene family defined by this probe, since it lacks only a few of the numerous hybridizing fragments (shown by arrows in Figure 2A). This places the Ars-A $V_H$ gene segment toward the proximal (3′) end of the J558 $V_H$ gene cluster with respect to $D_H$. Unexpectedly, 94B10 contains the full complement of J558 $V_H$ genes. Since 94B10 uses a 3660 $V_H$ gene, this suggests that the J558 $V_H$ family maps 5′ of the 3660 $V_H$ gene family with respect to $D_H$, a result in conflict with the accepted $V_H$ family order (Brodeur *et al.*, 1984; Riblet *et al.*, 1987). A probe derived from a 3660 $V_H$ gene revealed the presence of the rearranged 3660 $V_H$ gene and all of its family in 94B10, placing this $V_H$ gene at the 3′ end of its $V_H$ family in A/J (Figure 2B). However, the 3660 $V_H$ probe failed to hybridize to 91A3 DNA (Figure 2B) providing independent confirmation in two hybridomas that the J558 $V_H$ family is situated 5′ of 3660 within the $V_H$ complex in A/J.

The B cell lines from A/J, BALB/c and C57BL/6 were then probed with a fragment specific for the unmapped VGAM3-8 $V_H$ family. Figure 3 shows that the VGAM3-8 $V_H$ family is situated 3′ of the J558 $V_H$ family in all three strains and is positioned 5′ of 3660 in A/J. Fragments derived from the 3609, S107, J606, X24, Q52 and 7183 $V_H$ gene families were then used to probe the B cell lines from the three inbred strains. Figure 4A shows that with the exception of 3609, all other $V_H$ gene families are absent in 91A3 DNA, placing 3609 5′ of J558 while S107, VGAM-8, J606, X24, Q52 and 7183 all map 3′ of J558 in A/J mice.

In addition to the VGAM3-8 $V_H$ family, the J606 and S107 $V_H$ probes hybridized to *Eco*RI-digested 94B10 DNA placing these three $V_H$ gene families 5′ of 3660 and 3′ of J558 (data not shown but summarized in Table I). Mapped 3′ of 3660 were the X24, Q52 and 7183 $V_H$ familes since specific probes representing these three families failed to hybridize to the DNA in the two A/J hybrids (Figure 4A, Table I). Similar experiments were performed on the DNA of the BALB/c B cell lymphoma $BCL_1$ (Figure 4B), the BALB/c hybridoma 5G8 and the C57BL/6 $NP^b$-specific hybridoma S43.10 (data not shown but summarized in Table I). $BCL_1$ shows the same results as 91A3 since it has deleted seven $V_H$ gene families while retaining most of the 3609 and J558 $V_H$ families. Similarly 5G8 and S43.10 have deleted seven $V_H$ families (Table I). However, unlike the other lines tested, the C57BL/6 hybrid has also lost most of the 3609 $V_H$ gene family (Figure 5A, Table I). These results indicate that the rearranged J558 $V_H$ genes in the A/J and BALB/c lines are positioned 5′ of seven other $V_H$ family clusters, while in the C57BL/6 hybrid, the expressed J558 $V_H$ gene is 5′ of seven $V_H$ gene family clusters as well as the majority of the 3609 $V_H$ family.

*The 3609 and Q52 $V_H$ gene families are differentially interspersed in all three strains*

Of interest is the observation that a lightly hybridizing 3609-specific fragment present in A/J liver DNA is absent in 91A3 (see Figure 4A, marked with an arrow) and 94B10 (Table I). A longer autoradiographic exposure also failed to reveal this hybridizing fragment (data not shown). This suggests that at least one 3609 gene is interspersed and resides 3′ of both the J558 and 3660 $V_H$ gene families in A/J although it could conceivably reside on another chromosome which was independently lost in two separate fusions. $BCL_1$, while retaining most of the

**Table I.** Analysis of DNA of various B cell lines containing a single VDJ rearrangement with DNA probes for $V_H$ gene families

| Cell line | VH[1] | 3609 | J558 | S107 | VGAM3-8 | J606 | 3660 | X24 | Q52 | 7183 |
|---|---|---|---|---|---|---|---|---|---|---|
| 91A3 | (J558) e[2] | +[3] | +/−[6] | − | − | − | − | − | − | − |
| 94B10 | (3660) e | +[3] | + | + | + | + | + | − | − | − |
| $BCL_1$ | (J558) a | +[4] | +/−[6] | − | − | − | − | − | − | − |
| 5G8 | (J558) a | + | +/−[6] | − | − | − | − | − | − | − |
| S43.10 | (J558) b | +/−[5] | +/−[6] | − | − | − | − | − | −[7] | ND |

[1]Productively rearranged heavy chain variable region gene family.
[2]Haplotype designations are: e, A/J; a, BALB/c; and b, C57BL/6.
[3]At least one 3609 $V_H$ gene is absent.
[4]Four 3609 $V_H$ genes are absent.
[5]Three 3609 $V_H$ genes are present upstream to 186-2.
[6]Presence and absence of family members of the productively rearranged $V_H$ gene.
[7]One Q52 $V_H$ gene present.
ND, not done.

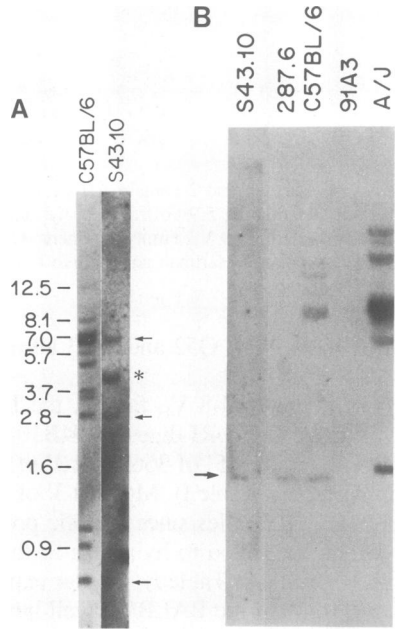


**Fig. 5.** Evidence that Q52 and 3609 $V_H$ genes are interspersed in C57BL/6. DNA from hybridomas haploid for the $V_H$ complex from C57BL/6 (S43.10 and 287.6) and A/J (91A3) were digested with *Eco*RI and probed with the 3609 (**A**) or Q52 (**B**) $V_H$ fragments described in Figure 3. Note the presence of a single 2.2-kb *Eco*RI fragment hybridizing to $V_H$ Q52 and three fragments hybridizing to the 3609 $V_H$ probe (arrows). Since the 3609 probe contains $J_H3-J_H4$ sequences, it hybridizes to the 4.8-kb rearranged $V_H$ gene in S43.10 (asterisk).

3609 $V_H$ family, has deleted four members of this $V_H$ gene family (Figure 4B). However, the hybridoma 5G8 appears to contain a full complement of 3609 (Table I). This would be expected if the dispersed 3609 genes were located between the rearranged J558 genes used by $BCL_1$ and 5G8. That is, in BALB/c, the 3609 and J558 $V_H$ gene clusters may overlap. The C57BL/6 hybridoma, S43.10, contains two examples where $V_H$ genes appear to be interspersed 5′ of their specific $V_H$ families. Three 3609-specific hybridizing fragments are present in this hybrid (Figure 5A). This hybrid also contains a Q52 $V_H$ gene that resides on a 2.2-kb *Eco*RI fragment interdigitated to a position 5′ of or within the J558 $V_H$ cluster (Figure 5B). Preliminary analysis of a second $NP^b$ producing hybrid, 287.6, also reveals the presence of this Q52 $V_H$ gene segment (Figure 5B).

## Discussion

Partial maps of the $V_H$ complex in three murine $V_H$ haplotypes were generated using deletion mapping. These maps show significant differences from the murine $V_H$ family organization inferred by a number of previous studies, many of which employed $Igh^e$ and $Igh^a$ recombinant inbred strains (Brodeur *et al.*, 1984; Riblet *et al.*, 1987). In A/J, all of the $V_H$ families have been mapped with respect to the J558 and 3660 $V_H$ families to generate a map order of:

$$3609-J558-(J606,VGAM3\text{-}8,S107)-3660-(X24,Q52,7183)-D_H.$$

The families in parentheses could not be ordered with respect to each other. In BALB/c and C57BL/6, eight $V_H$ families were positioned with respect to the J558 $V_H$ genes in both strains. BALB/c is similar to A/J in that most members of the 3609 $V_H$ family are situated 5′ of the rearranged J558 $V_H$ genes in both strains while the J606, VGAM3-8, S107, 3660, X24, Q52 and 7183 $V_H$ familes are 3′. The $V_H$ map of C57BL/6 resembles that of A/J and BALB/c in that the latter seven $V_H$ families are 3′ of its J558 $V_H$ rearrangement. However, C57BL/6 is distinct from the other two $V_H$ haplotypes in that the majority of its comparatively small 3609 $V_H$ family is situated 3′ of its rearranged J558 $V_H$ gene. Additionally, a Q52 $V_H$ gene segment is interspersed 5′ of the C57BL/6 186-2 J558 $V_H$ gene segment. Collectively, the results of this study generally correlate with work suggesting that normalized utilization frequencies of $V_H$ gene segments in VDJ rearrangements during B cell differentiation reflect their linear order in the $V_H$ complex (see below).

Although deletion mapping has previously been used to determine the organization of several kappa light chain variable region ($V_K$) families (Seidman *et al.*, 1980; Selsing and Storb, 1981; Heinrich *et al.*, 1984), limited use of this technique has been applied to the $V_H$ complex. Deletion mapping was used to position some J606 $V_H$ gene segments 5′ of S107 and the Q52 $V_H$ family 3′ of S107 in BALB/c (Cory and Adams, 1980; Kemp *et al.*, 1981). Another study, also in BALB/c, mapped S107 3′ of J558 using this technique (Rechavi *et al.*, 1982). These early studies are generally consistent with our findings. However, deletion mapping has not been more widely utilized probably due to the rarity of B cell lines and hybridomas that contain a single VDJ rearrangement. Most contain at least one additional $V_H$ complex that may be represented in germ-line configuration as a nonproductively rearranged allele and/or as a result of the fusion partner contribution. Due to the extreme difficulty in obtaining hybridomas that were suitable for deletion mapping experiments only five B cell lines representing three inbred strains

and one hybridoma from an F1 mouse were available for this study after extensive screening.

The B cell lines were required to fulfill two criteria in order to be utilized for deletion mapping experiments. The first was that the B cell lines contained a single VDJ rearrangement. The second criterion was that rearrangement and juxtaposition of the $V_H$ gene elements resulted in the loss of intervening DNA. Four of the B cell lines in this study contain rearranged $D_H$ elements that have been definitively characterized. The $D_H$ of 94B10 has not been sequenced. Nucleotide sequence analyses of other anti-arsonate antibodies that utilize the 3660 $V_H$ gene segment (Ars-C antibodies) in A/J as well as BALB/c have shown that Ars-C antibodies use DFL16.1 in their rearranged $V_H$ (Near *et al.*, 1984); serological studies of the 94B10 antibody have indirectly suggested that 94B10 utilizes DFL16.1 (Estess *et al.*, 1979; Milner and Capra, 1984). Since Ars-C $V_H$ sequences appear to be extremely homogeneous (Milner and Capra, 1982; Near *et al.*, 1984), 94B10, in all likelihood, utilizes DFL16.1 as well. Since no other DFL16, Dsp2 or DQ52 $D_H$ gene segments have been identified that reside 5′ of DFL16.1 in BALB/c, we concluded that testing for the presence or absence of 5′ flanking sequences of DFL16.1 was a valid criterion for eliminating inversion as a potential recombination mechanism. We note that, although eight B cell lines appeared to contain a single $V_HD_HJ_H$ rearrangement, and each of these had deleted DNA between $D_H$ and $J_H$, two of the lines retained sequences 5′ to DFL16.1 and thus could not be utilized in this study (unpublished observations). Collectively, the data suggest that the criteria described above should distinguish B cell lines that can be utilized for $V_H$ mapping purposes.

While the sample size of the B cell lines used in this study is limited, we believe that the information derived from these lines by deletion mapping reflects the relative positions of the $V_H$ families in the $V_H$ complex. There are a number of potential artifacts that could obscure the results in a given B cell line; however, one would have to argue that the identical artifacts occurred in five independently derived B cell lines and we consider the latter to be unlikely. Four of the B cell lines, representing three inbred strains, have rearranged a J558 $V_H$ gene; in each case, the results with respect to $V_H$ family order and $V_H$ gene segment interspersion are consistent. Additionally, the 94B10 hybrid provides independent confirmation that the J558 $V_H$ family maps 5′ of the 3600 $V_H$ family in A/J. Taken together, the data suggest that careful analyses of B cell lines for deletion mapping can provide a partial map of the $V_H$ complex.

The 3660 $V_H$ gene family has been mapped toward the 5′ end of the $V_H$ complex in the accepted $V_H$ map (Riblet *et al.*, 1986, 1987). We place it significantly more $D_H$ proximal in A/J and 3′ of J558 in BALB/c and C57BL/6. There is evidence for preferential expression of a 3660 $V_H$ gene in the BALB/c neonatal antibody response to 2,4-dinitrophenyl (Riley *et al.*, 1986) and preferential utilization of this $V_H$ gene in B cell lineages derived from fetal liver (Wu and Paige, 1986). Biased mRNA expression of the comparatively small X24 $V_H$ family (Brodeur and Riblet, 1984; Hartman and Rudikoff, 1984) as well as the J606 $V_H$ gene family is likewise observed in RNA colony blot assays of colony forming pre-B cells (CFU-B) from BALB/c and $BDF_1$ fetal liver (Wu and Paige, 1986). By contrast, message hybridizing to the 3609 $V_H$ gene family is the most infrequently expressed $V_H$ gene family in these two strains (Wu and Paige, 1986). However, the 3609 $V_H$ gene family is more frequently utilized in C57BL/6 (Wu and Paige, 1986). The present study indicates that the majority of the 3609 $V_H$ gene family are located 3′ of the J558 $V_H$ family in the C57BL/6 hybridoma S43.10 (Figure 5A). The more precise mapping of 3609 interdigitation positions an interspersed 3609 $V_H$ gene segment 3′ of the 3660 $V_H$ family in A/J (Figure 4A). The results of Wu and Gilliam (1986) may therefore indicate that one or more of the interspersed C57BL/6 3609 $V_H$ gene segments is significantly $D_H$ proximal. Taken together, the evidence suggests that differences may exist with respect to the $V_H$ family organization in the three inbred strains and/or that apparent variations within the $V_H$ map are perhaps more subtly reflected by the positions of interspersed $V_H$ family members within the $V_H$ complex.

The high utilization frequencies of the J558 $V_H$ family (Yancopoulos *et al.*, 1984; Perlmutter *et al.*, 1985; Wu and Paige, 1986) would seem to contradict the $V_H$ order presented herein. However, since recent evidence suggests that the J558 $V_H$ family contains 500−1000 members (Livant *et al.*, 1986), the seeming over representation of this $V_H$ family is probably due to sampling (Yancopoulos and Alt, 1986; Wu and Paige, 1986). Thus, if preferential rearrangement of $V_H$ gene segments in *in vitro* B cell differentiation or immature *in vivo* systems reflects their proximity to a rearranged $D_HJ_H$, then the $V_H$ map implied by the proximal $V_H$ gene segment usage model of $V_H$ gene segment rearrangement is more consistent with the $V_H$ maps generated in our study than the $V_H$ gene family organization generated previously (Brodeur *et al.*, 1984; Riblet *et al.*, 1986, 1987). The $V_H$ family order in the present study is also consistent with the notion that the recombinase that mediates $V_H-D_HJ_H$ rearrangement during B cell differentiation may operate by a one-dimensional tracking mechanism (Wood and Tonegawa, 1983; Yancopoulos *et al.*, 1984).

Previous work suggested that related $V_H$ genes in the inbred strains are organized into non-overlapping clusters (Kemp *et al.*, 1981; Givol *et al.*, 1981; Crews *et al.*, 1981; Rechavi *et al.*, 1982). That some $V_H$ gene families can be partially characterized in this manner is generally supported by our studies. However, our data suggests that at least some $V_H$ families are organized into irregular clusters. This is supported by the observation that some $V_H$ gene segments in A/J, BALB/c and C57BL/6 are interspersed with members of another $V_H$ gene family, implying the potential for other $V_H$ family members to be similarly interdigitated. Indeed Kemp *et al.* (1981) showed that a $V_H$ gene segment that in retrospect appears to hybridize in a pattern uncharacteristic of the J606 $V_H$ family in BALB/c was located ~14 kb 5′ of a $V_H$ gene whose sequence clearly places it in the J606 $V_H$ gene family. More recent studies show that the Q52 and 7183 $V_H$ gene families are interdigitated in the NFS/N inbred strain and NIH Swiss outbred mice (Kleinfield *et al.*, 1986; Reth *et al.*, 1986). In the present study, at least one 3609 $V_H$ gene in A/J, three in C57BL/6 and four in BALB/c do not reside within their respective $V_H$ family clusters. In addition to the interspersion of the 3609 $V_H$ family in C57BL/6, a Q52 $V_H$ gene is interdigitated 5′ of or within the J558 $V_H$ family cluster in this strain. This Q52 $V_H$ gene segment is present in the germline of 14/18 strains assayed by Southern filter analysis (Brodeur and Riblet, 1984), yet it is lost in two independently derived A/J hybridomas and a BALB/c B cell lymphoma that utilize upstream $V_H$ genes. Both lines of evidence argue for the presence of this gene within the $V_H$ locus as opposed to being interspersed to another chromosome as has been reported for some human $V_\kappa$ genes (Lotschen *et al.*, 1986). Thus, data from four indepen-

dent $V_H$ haplotypes as well as from NIH Swiss mice indicate that some $V_H$ family interdigitation may be a common phenotypic trait of the murine $V_H$ complex.

The apparent clustering of some murine $V_H$ and $V_x$ (Henrich *et al.*, 1984) genes into specific families is unlike the situation observed in both human (Kodaira *et al.*, 1986) and rabbit (Gallarda *et al.*, 1985), in which extensive familial interdigitation seems to be a more general phenomenon. Similarly, human $V_x$ genes have been shown to be interdigitated with respect to subgroups (Pech *et al.*, 1984; Pohlenz *et al.*, 1987). The extensive inbreeding of mice required to produce the inbred strains may have generated a distorted view of the murine $V_H$ complex that is reflected by familial clustering of many $V_H$ gene segments; alternatively, $V_H$ and $V_x$ clustering may be a genetic trait of the *mus* genus. An examination of the organization of these loci in wild mice should prove interesting in this regard. The clustering of related $V_H$ genes could have occurred by unequal crossovers into specific regions of the $V_H$ locus that contained a high density of related $V_H$ gene segments and flanking sequences. If there is a significant difference in the organization of the $V_H$ locus in the various inbred and recombinant inbred strains, this would indicate that the $V_H$ locus may be more dynamic than previously believed, with a capacity to reorganize significant sections of $V_H$-containing DNA. $V_H$ family shuffling could be generated by crossovers (Hood *et al.*, 1975), hotspots of recombination (Riblet, 1977; Steinmetz *et al.*, 1982, 1986), multiple reciprocal recombinations, inversions of DNA segments containing one or more $V_H$ families, or by a combination of any of these mechanisms.

## Materials and methods

*DNA*

A/J and BALB/c liver DNA as well as DNA from the 91A3 and 94B10 hybridomas were prepared as described previously (Blin and Stafford, 1976). The $BCL_1$ B cell lymphoma DNA (Gronowicz *et al.*, 1980) was kindly provided by Dr Y.-W.Chen. The hybridoma DNA from S43.10 and 284.6 were kindly provided by Drs C.Kocks and K.Rajewsky. S43.10 is a subclone of the NP-specific C57BL/6 hybrid S43 (Reth *et al.*, 1978) that was selected for loss of the Ig-expression of the fusion partner (G.Wildner and K.Rajewsky, unpublished). 5G8 is a BALB/c anti-*Haemophilus influenzae* type B lipooligosaccharide hybridoma generated by Dr E.Hansen. 287.6 is a hybridoma generated by Dr G.Wildner from a (CBA × C57BL/6) F1 mouse that uses the same J558 $V_H$ gene as S43.10 and expresses the $NP^b$ idiotype (M.Siekevitz, C.Kocks, K.Rajewsky and R.Dildrop, submitted). The C57BL/6 liver DNA was kindly supplied by Dr T.Blankenstein.

*Probes*

The 5′ DFL16.1$^e$ probe is a 270-bp *Ava*II fragment containing 5′ flanking sequences of DFL16.1$^e$ (Landolfi *et al.*, 1986). The 3′ DFL16.1$^e$ probe is a 700-bp *Bam*HI–*Ava*II fragment containing 3′ flanking sequences of DFL16.1$^e$ (Landolfi *et al.*, 1986). The $J_H1$ probe is a 600-bp *Bam*HI–*Pst*I fragment containing $J_H1$ and 5′ flanking sequences. A 1.3-kb *Bam*HI–*Eco*RI fragment contains the $J_H3$ and $J_H4$ segments. $V_H$ family-specific probes were isolated as a 1.3-kb *Bam*HI–*Eco*RI fragment from $V_H$ 3609P (Riblet *et al.*, 1986), a 270-bp *Pst*I fragment from the cDNA 93G7 (Sims *et al.*, 1982) that hybridizes to the J558 $V_H$ gene family, a 600-bp *Eco*RI–*Xba*I fragment from M460 that is specific for the 3660 $V_H$ family (Dzierzak *et al.*, 1985), a 500-bp *Xho*II fragment from VGAM3-8 that hybridizes to the VGAM3-8 $V_H$ family (Winter, *et al.*, 1985), a 450-bp *Pst*I fragment from S107 (Crews *et al.*, 1981), a 600-bp fragment from pBV14 that detects the J606 family, a 500-bp *Eco*RI–*Pst*I fragment from $V_H$ 39 that hybridizes to the X24 family (Hartman and Rudikoff, 1984), a 320-bp *Eco*RI–*Hin*dIII fragment from $V_H$Q52 (Brodeur and Riblet, 1984), and a 280-bp *Pst*I–*Eco*RI fragment from $V_H$ 81X that recognizes the 7183 $V_H$ gene family (Yancopoulos *et al.*, 1984).

*Southern filter hybridization*

Fifteen micrograms of liver or B cell DNA was digested with the indicated restriction endonuclease (Boehringer Mannheim), applied to an 0.8% agarose gel and electrophoresed in the presence of 0.094 M Tris, pH 8.0, 0.02 M sodium citrate, 0.0175 M NaCl and 0.2 mM ethylenediaminetetraacetic acid at 85 mAmps for 16–18 h. The size-fractionated DNA was then transferred to nitrocellulose (Schleicher and Schuell) and pre-hybridized in the presence of 50% formamide (BRL) as described (Sims *et al.*, 1982). Probes were radiolabeled by nick translation (BRL) to a specific activity of $2 \times 10^7 - 5 \times 10^8$ c.p.m./μg and denatured by boiling for 7 min prior to addition to fresh pre-hybridization solution. Hybridization proceeded for a minimum of 16 h at 42°C. Nitrocellulose filters were then washed at 42°C in 0.015 M NaCl, 0.0015 M sodium citrate and 0.1% SDS for 1 h. Molecular weights were computed utilizing λ CB2 digested with *Eco*RI and/or wild-type λ digested with *Hin*dIII.

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