THE ORGANIZATION OF THE MOUSE *Igh-V* LOCUS

Dispersion, Interspersion, and the Evolution of V_H Gene Family Clusters

By PETER H. BRODEUR, GAMAL E. OSMAN, JAMES J. MACKLE, AND THOMAS M. LALOR

From the Immunology Program, Sackler School of Graduate Biomedical Sciences and the Department of Pathology, Tufts University School of Medicine, Boston, Massachusetts 02111

Studies of Ig gene structure and organization during the past decade have illuminated the central mechanisms of antibody diversification: the somatic rearrangement and reassortment of multiple gene segments, junctional flexibility, and point mutation. The inherited set of Ig gene segments provides a diversified genetic basis upon which these dynamic processes operate during ontogeny. Thus, the composition of these germline genes imposes a major influence on the development of the antibody repertoire.

The present study examines the germline content and organization of the mouse heavy chain variable region genes (V_H genes). We set out to analyze the locus in sufficient detail and resolution to provide the basis for determining the extent of inherited *V* gene diversity, the evolution of the germline repertoire, and any functional consequences of the physical arrangement of the V_H gene segments.

The mouse *Igh* locus consists of at least 100-200 V_H genes (1-3). V_H gene families, defined by nucleotide sequence relationships, comprise distinct sets of highly related V_H genes that can be identified by hybridization using prototypic V_H gene probes. This classification of V_H gene families (1, 4) has provided a useful framework for the study of germline V_H gene content, polymorphism, and utilization (1-7). Whereas the general organization of the *Igh* locus is 5'-V_H-D-J_H-C_H-3' (8, 9), previous studies of V_H gene family organization have resulted in partial, relatively low resolution maps lacking consistency between reports (10-12).

"Deletion mapping" takes advantage of the fact that V_H gene rearrangements result in the deletion of DNA originally separating the rearranged V_H gene segment and the D-J_H-CH region (13). We have constructed a panel of 32 pre-B cell lines, most of which have rearranged V_H genes on both chromosomes. Since these cell lines were derived from F_1 mice heterozygous at the *Igh* locus, V_H gene deletions can be identified using RFLPs. V_H gene analyses of 51 independently rearranged chromosomes are consistent with a single V_H gene map order of nine V_H gene families. The genomic stability of these cell lines and consistent deletion profiles of all 51 rearranged loci provide a high resolution V_H gene map that has compelling experimental support.

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Materials and Methods

Mice. BALB/c.Ann.*xid* mice were obtained from Dr. Carl Hansen (National Institutes of Health, Bethesda, MD). BALB/cJ and C57BL/10J mice were obtained from The Jackson Laboratories, Bar Harbor, ME. C.AL-20 mice were provided by the late Dr. Frances Owen (Tufts Medical School).

Cell Lines. "CXXB" cell lines were generated using bone marrow cells from 8-wk-old (BALB/cAnn.*xid* × C57BL/10J)F_1 mice. Cells were transformed in vitro using Abelson Murine Leukemia Virus (A-MuLV)[1] and were initially cloned in soft agar as described by Rosenberg and Baltimore (14). Some lines were subsequently subcloned by limiting dilution in 96-well plates. Cells were grown in RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented to contain 10% FCS (HyClone Laboratories, Logan, UT), 25 mM Hepes, pH 7.2 (Sigma Chemical Co., St. Louis, MO), 5×10^{-5} M 2-ME, and penicillin/streptomycin (Gibco Laboratories). The CXAL 1.40 cell line was transformed as above from bone marrow cells of (BALB/c × C.AL-20)F_1 mice. Although we have not analyzed these Abelson virus-transformed cell lines by immunofluorescence, based on previous studies of A-MuLV-transformed adult bone marrow cells, we assume that they are of the pre-B cell phenotype, i.e., cytoplasmic μ^+ and surface IgM^-.

The plasmacytoma PC3609 was induced in an NZB mouse by Dr. Martin Weigert, Fox Chase Cancer Center, Philadelphia, PA (15) and provided to us as a solid tumor. Before use in our studies, we adapted PC3609 to tissue culture using standard protocols. C3a is an LPS-dependent transformed B cell line of BALB/c origin (16).

Southern Blots. High molecular weight genomic DNA was prepared as described (1). DNA preparations were digested to completion with 3–5 U of restriction endonuclease per microgram. DNA for 5–12 h at 37° C using buffers recommended by the manufacturer (Bethesda Research Laboratories, Gaithersburg, MD). Digested DNA samples (10 μg/lane) were fractionated through 0.7% agarose gels (20 cm × 24 cm, 300 ml) at 45 V for 16–20 h in TAE buffer (40 mM Tris, 20 mM sodium acetate, 2 mM EDTA, pH 7.8). Fractionated DNA was transferred to nylon membranes (Nytran; Schleicher & Schuell, Inc., Keene, NH) by either electroblotting (25 V, 12–16 h in 0.25 × TAE) or by capillary transfer using 20× SSC (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate). Blots were baked at 80° C for 2 h and hybridized with ^{32}P-labeled DNA probes (17) as described (1). After hybridization, filters were washed twice at 68°C in 3× SSC, 0.2% SDS for 15 min and once in 0.2× SSC and 0.1% SDS for 30 min. Filters were exposed to KODAK XAR-5 film at −80°C using an intensifying screen (Lighting-Plus; DuPont Co., Wilmington, DE).

Probes. The hybridization probes used in this study were agarose gel-purified DNA fragments: V_H7183, 1.1-kbp Eco RI–HaeIII fragment from pVHSAPC-15 (1); V_HQ52, 250-bp Hha I fragment from pV_HQ52 (1); V_HS107, 445-bp Pst I fragment from pV1 (18); V_HX24, 500-bp Eco RI fragment from p17 (19); VGAM3.8, 4.0-kbp Eco RI fragment (CBA germline) cloned in λgtWES; V_H36-60, 700-bp Hind III–Eco RI fragment from pRN5.15 (20); V_HJ606, 600-bp Bam HI–Eco RI fragment from pBV14J606 (1); V_H3609, 1.5-kbp Eco RI–Bam HI fragment (BALB/c germline); V_HJ558, 580-bp Eco RI fragment from pVA1 (21); J_H, 1.9-kbp Bam HI– Eco RI fragment from pJ_{11} (22); β_2 microglobulin, 1.0-kbp Hind III–Eco RI fragment (23).

Cloning and DNA Sequencing. The two rearranged J_H hybridizing fragments from cell line CXAL 1.40 were cloned in λgtWES and recombinant phage were identified using the pJ_{11} probe. V_H containing fragments were subcloned in the plasmid pUC-18. The V_HJ558-bearing fragments (see Results) were subcloned into M13 phage and sequenced using standard dideoxynucleotide sequencing procedures (24).

Densitometry. Films of Southern blots hybridized with the V_HS107 probe were analyzed using a densitometer (model 620; Bio-Rad Laboratories, Richmond, CA). The copy number of the monomorphic germline V1 gene was determined by comparing the ratio of the densities of V1 bands with polymorphic V_HS107 family Eco RI fragments in BALB/c, C57BL/10, (BALB × C57BL)F_1, and CXXB cell line DNA samples.

[1] *Abbreviation used in this paper:* A-MuLV, Abelson murine leukemia virus.

Results

Pre-B cell lines were generated by in vitro transformation of adult bone marrow cells from F_1 mice carrying the *Igh*^{a} and *Igh*^{b} parental haplotypes. These (BALB/c Ann.*xid* × C57BL/10)F_1 cell lines are designated with the prefix "CXXB."

DNA from ~100 independent CXXB cell lines were initially screened by Southern blot hybridization with probes for the two V_H gene families previously mapped most proximal to the D segments, V_HQ52 and V_H7183 (10). 29 CXXB cell lines, most of which have deleted the V_HQ52 or V_H7183 families from one or both chromosomes, were analyzed for the presence or absence of nine V_H gene families.

Southern blots hybridized with probes for J_H and the nine V_H families are presented here for 17 CXXB cell lines (Figs. 1 and 2). Southern blot data from all 29 CXXB cell lines analyzed are presented in summary form (Figs. 3 and 4).

CXXB Cell Lines are Independent, Clonal, and Stable. Fig. 1 is a Southern blot of CXXB cell line DNA samples hybridized with the J_H probe. No two lanes show identical patterns of rearranged *Igh* loci, demonstrating the independence of these transformed cell lines. 15 of the cell lines have two rearranged J_H hybridizing Eco RI fragments, while two cell lines, F52 and F2, each have a single rearranged J_H hybridizing fragment. None of the cell lines have retained an unrearranged, germline *Igh* locus (Fig. 1). Moreover, there is no evidence for further *Igh* locus rearrangement during culture; that is, no additional faintly hybridizing J_H fragments were observed during in vitro propagation of these lines. We conclude that the CXXB cell lines analyzed here are clonal cell populations with stably rearranged heavy chain loci.

Construction of a V_H Gene Map. Nine Southern blots, each with DNA from 17 CXXB cell lines and liver DNA from strains of the parental *Igh* haplotypes (BALB/c

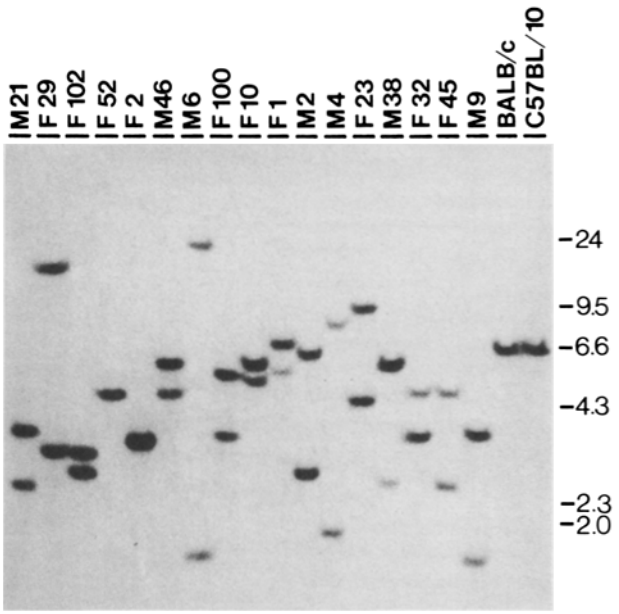

FIGURE 1. Abelson virus-transformed cell lines (CXXB) are clonal, and have stably rearranged *Igh* loci. Eco RI-digested DNA from CXXB cell lines or liver (BALB/c and C57BL/10) was electrophoresed through a 0.7% agarose gel, transferred to a Nytran membrane, and hybridized with the J_H probe (pJ_{11}). Size markers are based on Hind III-digested λ DNA.

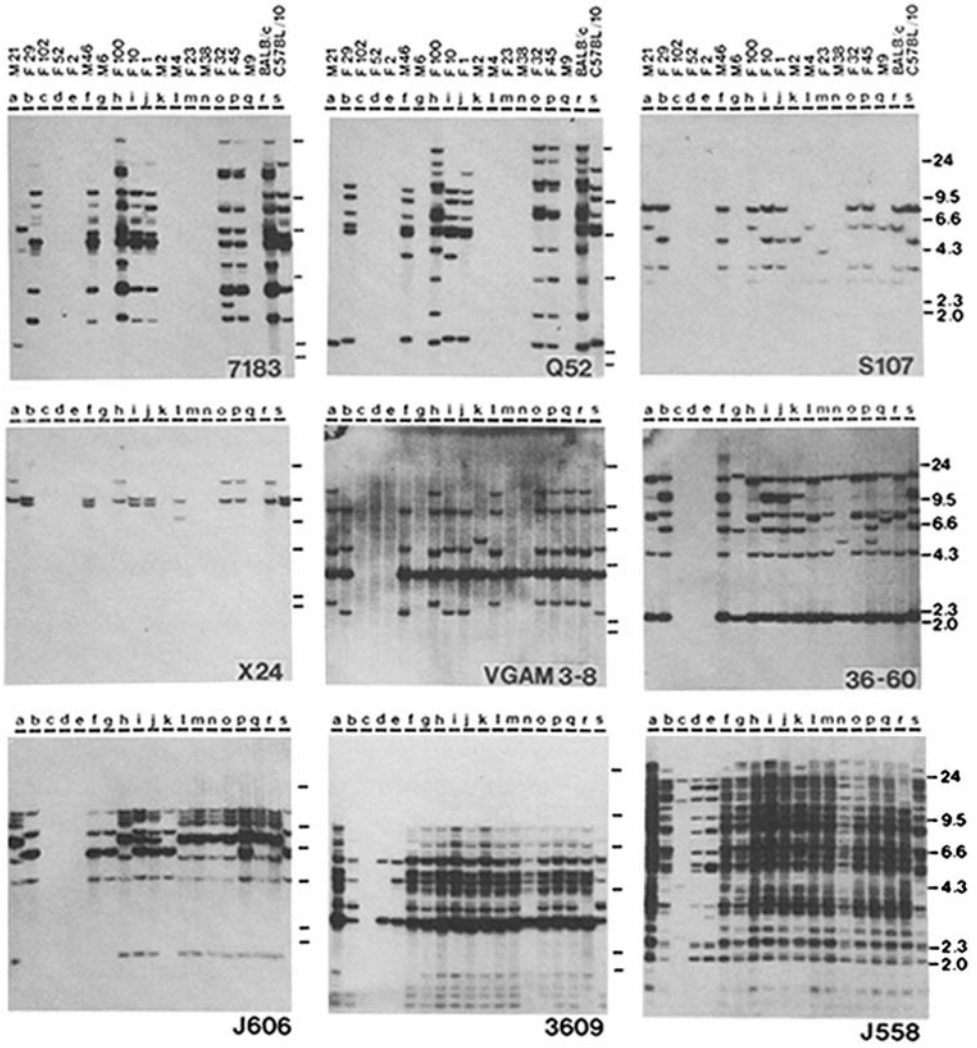

FIGURE 2. V_H gene analysis of 17 CXXB pre-B cell lines. DNA from indicated cell lines or liver (BALB/c and C57BL/10) was digested with restriction endonucleases, electrophoresed through 0.7% agarose gels, and transferred to Nytran membranes. Blots were hybridized with the indicated V_H gene probe. All Southern blots are of Eco RI-digested DNA except V_HX24 (Pst I) and VH36-60 (Hind III). These data are summarized in Fig. 3

and C57BL/10), were hybridized with V_H gene probes. As shown in Fig. 2, some cell lines have deleted two or more V_H gene families from both chromosomes, for example, nine lines (F102, F52, F2, M6, M2, M4, F23, M38, and M9) have deleted all members of the V_H7183 and VHQ52 gene families. Other cell lines have deleted VH7183 and VHQ52 on one chromosome only, for example, F100 has VH7183 and VHQ52 patterns identical to the *Igh*^{a} (BALB) parental strain but has deleted all VH7183 and VHQ52 hybridizing fragments that are *Igh*^{b} (C57BL) specific. Similarly, CXXB F_1 has deleted its *Igh*^{a} V_H7183 and V_HQ52 families but retained V_H7183 and V_HQ52 patterns identical to those of the germline *Igh*^{b} haplotype.

In many instances, a particular V_H family is partially deleted from one (or both) alleles. For example, examination of the V_HS107 pattern of CXXB M9 (Fig. 2) reveals the deletion of all but two germline Eco RI fragments.

The Southern blot data shown in Fig. 2 are summarized in Fig. 3; closed boxes indicate that the genes of that particular family are all in germline configuration, while open boxes indicate the deletion of the entire family. Partial deletion of a family is denoted by a half-filled box. The V_H gene haplotype of each allele, *Igh*^{a} and *Igh*^{b}, is indicated for each cell line.

The analysis of deletions in the 29 CXXB cell lines (47 V_H rearranged chromosomes) has yielded a relative chromosomal order of V_H genes that is consistent with all observed patterns of V_H gene deletion and retention. These deletion profiles are summarized in Figs. 3 and 4.

V_HQ52 and V_H7183 Family Members are Interspersed. Analyses of *Igh*-recombinant mouse strains have shown that the V_HQ52 and V_H7183 families are the most D-proximal V_H gene families (10) and interspersion of these two families has been reported for NIH/Swiss (25) and NSF/N (26) strains. The CXXB cell lines show that V_HQ52 and V_H7183 family members are extensively interspersed in both *Igh*^{a} and *Igh*^{b} haplotypes. Partial deletion of both families was observed for nine cell lines; five having deletions on the *Igh*^{a} allele (M21, F32, F45, M8, M52) and four having deletions on the *Igh*^{b} allele (F29, M46, F10, M30). Most of these nine chromosomes have deleted one or a few germline restriction fragments from each family, indicating interspersion of the most 3′ (D-proximal) V_HQ52 and V_H7183 genes. Notably, CXXB M21 has rearranged a V_H7183 gene resulting in the deletion of most V_H7183

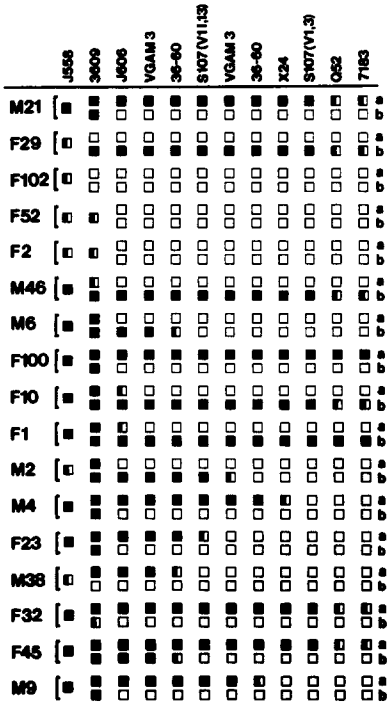

FIGURE 3. Summary of V_H gene content of CXXB cell lines. BALB/c "a" and C57BL/10 "b" alleles are indicated for each cell line. (■) Germline V_H gene pattern present; (□) deletion of germline V_H genes; (◧) partial deletion of germline V_H genes. The single boxes shown for V_HJ558 indicate the difficulty in distinguishing polymorphism in this complex family (see text). CXXB F52 and F2 have retained only monomorphic V_H3609 fragments. Copy numbers of monomorphic V_HS107 fragments, V1 and V3, were determined for each cell line by densitometry (see Materials and Methods). Data are from Fig. 2.

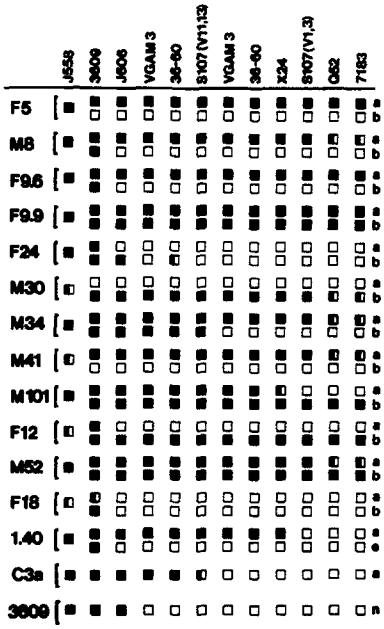

FIGURE 4. Summary of V_H gene content in pre-B and B cell lines. All cell lines are from the CXXB except for CXAL 1.40, C3a (BALB/c), and PC3609 (NZB plasmacytoma). For details, see Fig. 3

and V_HQ52 germline fragments (Figs. 1 and 2). Since CXXB M21 has retained a single germline V_HQ52 fragment and two germline V_H7183 fragments, we conclude that the most 5′ members of these two families are also interspersed. Therefore, members of these two families are extensively interspersed and together occupy the same physical subregion of the *Igh* locus.

V_HS107 Family Members are Dispersed. The V_HS107 family consists of four genes in the BALB/c genome (18). One member, V1 (18), is the V_H gene encoding in the T15 idiotype bearing antiphosphorylcholine antibodies that predominate in the BALB/c primary response (27). The two other functional members of the BALB/c V_HS107 family are V11 (5.7-kbp Eco RI) and V13 (2.8 kbp Eco RI). The V3 gene (3.4-kbp Eco RI) has several defects (28), rendering it nonfunctional.

C57BL/10 mice also have three functional V_HS107 genes and a pseudogene. The V1 allele of the *Igh*^{b} haplotype is also present on a 7.8-kbp Eco RI fragment (29). The Eco RI Southern blot patterns of BALB/c and C57BL/10 have three monomorphic fragments corresponding to the BALB/c V1, V3, and V13 genes as well as one polymorphic fragment (V11 in BALB/c). With a slight increase in the hybridization stringency for V_HS107-probed Southern blots, the C57BL/10 V13-like fragment, which comigrates with the BALB/c V13 gene, is not detected. Therefore, V_HS107 gene analysis was performed using the increased hybridization stringency to allow a haplotype specific determination of the retention or deletion of the BALB/c V13 gene (Fig. 2).

The V_HS107 family of BALB/c maps as two distinct pairs (V3/V1 and V13/11), the V3/V1 pair mapping more proximal to the D segments and being the nearest V genes identified 5′ of the V_HQ52/V_H7183 cluster. For example, both CXXB M4 and M101 have rearranged the 3′ member of the two-gene VHX24 family, and have

deleted V1 and V3 but not V11 and V13. CXXB M9 has rearranged a V_H36-60 gene and also deleted V1/V3, but has retained V11/V13. CXXB F23 has retained two V_HS107 genes, a germline V13 gene, and a rearranged gene that is presumably V11. These results are consistent with a map order of 5'-V13-V11-3'.

Within the *Igh*^{b} haplotype the V_HS107 family is also dispersed, as shown by the rearrangement of a VGAM 3.8 member (CXXB M2), which resulted in the deletion of only two of the three *Igh*^{b} V_HS107 fragments (Figs. 2, 3).

The V_HX24 Family Maps 5' of V_HS107 V3-V1. The V_HX24 family consists of two genes in both BALB/c and C57BL/10 mice (19). Two independent cell lines, CXXB M4 and CXXB M101, have each rearranged a V_HX24 gene on the BALB/c chromosome. In both cases, these rearranged *Igh* loci have deleted V_HS107 V3/V1 and the entire V_HQ52/V_H7183 cluster without any detectable alteration in the germline patterns of the other seven V_H gene families. That both V_HX24 members are located between V_HS107 V13/V11 and V3/V1 is shown by the CXXB F23 and C3a cell lines (Figs. 2-4), which have each rearranged the V_HS107 V11 gene and deleted both members of the V_HX24 family. Furthermore, CXXB M9 has rearranged one of the two V_H36-60 genes, which also map between V13/V11 and V3/V1 on the BALB/c chromosome, and has deleted both V_HX24 genes. Therefore, the two V_HX24 genes show no evidence of being dispersed.

The V_HX24 genes also map between V_HS107 members in the *Igh*^{b} haplotype since CXXB M2 has deleted two V_HS107 fragments (including V1), as well as both V_HX24 genes, but has retained the 5.4-kbp V_HS107 fragment on the C57BL/10 chromosome.

The V_H36-60 Family Members Map to Three Locations. The VH36-60 family (20) consists of six and seven Hind III fragments for the *Igh*^{a} and *Igh*^{b} haplotypes, respectively. Two of the *Igh*^{a} fragments and one *Igh*^{b} fragment map between V_HS107 V13/V11 and V_HX24, whereas the majority of the V_H36-60 family resides 5' of the V_HS107 V13/V11 cluster. For example, the CXXB F23 (Figs. 3 and 5) and C3a (Fig. 4) cell lines have each rearranged the V_HS107 V11 gene (*Igh*^{a} allele) and have deleted the two V_H36-60 3' members (the smaller Hind III fragment in the doublet at 12-kbp and the smaller Hind III fragment in the doublet at 7.5 kbp). The V_H36-60 genes retained in CXXB 23 and C3a makeup the 5' V_H36-60 cluster, whereas the two fragments deleted by the V_HS107 V11 rearrangement make up the 3' V_H36-60 cluster of the BALB/c chromosome. Rearrangement of one of these 3' V_H36-60 genes (CXXB M9, Figs. 2 and 3) resulted in the deletion of V_HS107 V3/V1, V_HX24, V_HQ52, and V_H7183 families but the V_HS107 V13/V11 cluster and 5' V_H36-60 clusters were retained.

The *Igh*^{b} haplotype exhibits similar dispersion of V_H36-60 members since the cell line CXXB M2 has rearranged a VGAM3.8 gene and deleted a single Hind III fragment (9.5 kbp) from the germline V_H36-60 pattern of C57BL/10 (Figs. 2 and 3).

As expected from the above results, rearrangement of a member of the 5' cluster of V_H36-60 genes (CXXB M38, M6, and F45, Figs. 2 and 3) deletes the entire V_HS107 family.

The V_H gene predominately expressed in the antiarsonate response of BALB/c mice, V_H1210.7, is a V_H36-60 family member and is located on a 2.4-kbp Hind III fragment in both the BALB/c and C57BL/10 genomes (20). However, the V_H1210.7 gene resides on Eco RI fragments that are polymorphic between these two strains.

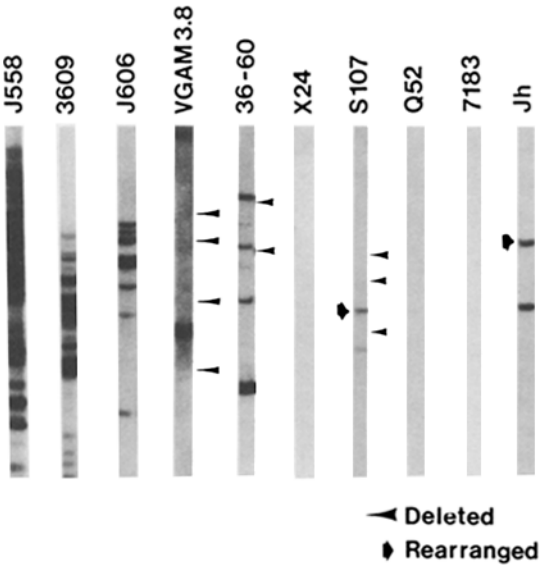

FIGURE 5. V_H gene deletion profile of cell line CXXB F23. DNA from CXXB F23 was digested with restriction endonucleases, electrophoresed through 0.7% agarose, and transferred to Nytran membranes. Each lane was hybridized with the indicated V_H gene probe. All lanes are of Eco RI-digested DNA except V_H36-60 (Hind III) and V_HX24 (Pst I). Deleted germline fragments are indicated by left-to-right arrows while rearranged fragments are shown by right-to-left arrows. Except for V_HJ558 and V_H3609, only genes from the *Igh*^{a} allele are present in CXXB F23 (see Fig. 3). Since individual lanes are from different gels, precise size comparisons cannot be made between families.

Southern blots of Eco RI-digested DNA (data not shown) show that the V_H1210.7 gene is not deleted from chromosomes that have rearranged other members of the V_H36-60 5′ cluster (CXXB M6, M38, F45), but is deleted from alleles that rearranged V_HJ606 genes (CXXB F10, F1). The V_H1210.7 gene, therefore, is one of the most 5′ members of V_H36-60. For example, CXXB M6 has rearranged a V_H36-60 gene and retained only two germline members of the family, one of which is V_H1210.7. CXXB F24 has a single rearranged V_H36-60 fragment and no germline V_H36-60 fragments. Based on the intensity of hybridization (the V_H36-60 probe used has 100% sequence identity with V_H1210.7) and the data presented above, it is likely that the rearranged gene in CXXB F24 is V_H1210.7. That the most 5′ V_H36-60 member is the germline V_H1210.7 gene is consistent with the size of the V_H36-60 hybridizing rearranged Hind III fragment in CXXB F24 (2.7 kbp) which is the predicted size of a V_H1210.7 gene rearranged to J_H4 (20).

As shown below, the most 5′ member of the V_H36-60 family is separated from the remainder of 5′ V_H36-60 cluster by at least one VGAM 3.8 gene. Therefore, members of the V_H36-60 family are located in at least three noncontiguous clusters.

VGAM3.8 Genes Map to Two Distinct Regions. The VGAM3.8 family of BALB/c consists of five Eco RI fragments (2). The germline 4.0-kbp fragment does not map in the same region as the remainder of the family as evidenced by deletions (*Igh*^{a} allele) in cell lines CXXB F23, C3a, and M38. CXXB F23 has rearranged V_HS107 V11 (*Igh*^{a} allele), deleting all VGAM3.8 members except the fragment at 4.0 kbp (Figs. 2, 5). The BALB/c-derived cell line C3a (16) has also rearranged V_HS107 V11 (Picarella, D., and N. Rosenberg, personal communication) and has similarly deleted all VGAM3.8 members except the 4.0-kbp fragment (Fig. 4). CXXB M38 has rearranged a 5′ V_H36-60 member and also retains only the 4.0-kbp VGAM3.8 fragment (Figs. 2, 3). Similarly, CXXB M6 has rearranged a 5′ V_H36-60 gene on the *Igh*^{b} allele and deleted all VGAM3.8 members except the 4.0-kbp germline Eco RI frag-

ment (Figs. 2, 3). Therefore, in both BALB/c and C57BL/10 strains, the 4.0-kbp Eco RI VGAM3.8 fragment maps to a distinct 5′ subregion of the *Igh* locus relative to the remainder of that family.

The 5′ VGAM3.8 gene(s) of BALB/c and C57BL/10 mice (4.0-kbp Eco RI fragment) can be distinguished by polymorphic Bgl II restriction sites (data not shown). Southern blot analysis of Bgl II–digested DNA maps the 5′ VGAM3.8 fragment between the V_HJ606 (CXXB F10,F1) and 5′ V_H36–60 clusters (CXXB M6, M38, F45). CXXB F24 has rearranged the most 5′ member of the V_H36–60 family and has deleted all members of VGAM3.8. Therefore, the VGAM3.8 family has a single dispersed fragment that maps between the two most 5′ members of V_H36–60.

Most of the VGAM3.8 family, however, maps between V_HS107 V13/V11 and the 3′ V_H36–60 cluster. CXXB M2 has rearranged a VGAM3.8 gene, deleting all germline VGAM3.8 fragments except the single VGAM3.8 Eco RI fragment at 4.0 kbp. Both CXXB M2 and M34 retain the germline V_HS107 V11 fragment but delete the 3′ members of V_H36–60. CXXB M9 has rearranged a 3′ member of V_H36–60 (deleting both germline 3′ V_H36–60 fragments on the BALB/c chromosome), but has retained the entire VGAM3.8 family. Taken together, these results demonstrate that the 3′ VGAM3.8 cluster maps between V_HS107 V11 and the two 3′ V_H36–60 fragments.

V_HJ606 Maps 5′ of V_H36–60. CXXB F10 and F1 have both rearranged a member of the V_HJ606 family (*Igh^a* allele), in each instance deleting two V_HJ606 members (10 and 5 kbp). In both cell lines, the entire V_H36–60 family is deleted, including the BALB/c $V_H1210.7$ gene-bearing fragment (Eco RI blot, data not shown). Rearrangement of the most 5′ V_H36–60 gene in CXXB F24 did not delete V_HJ606 fragments but resulted in the deletion of the entire VGAM3.8, V_HS107, V_HX24, V_HQ52, and V_H7183 families (Fig. 4). Therefore, the V_HJ606 cluster maps 5′ of all V_H36–60 members.

V_H3609 Maps 5′ of V_HJ606. Two cell lines of the CXXB panel have deleted the BALB/c V_HJ606 family and retained the BALB/c V_H3609 family (M6 and M2). Seven cell lines have C57BL/10 chromosomes with an intact V_H3609 family but no V_HJ606 members (CXXB M21, F100, M4, F23, M9, M8, F9.6). CXXB F2 has deleted all but four V_H3609 fragments and CXXB F52 has retained two V_H3609 fragments. Since the nondeleted fragments are monomorphic, it is not possible to assign them to either the BALB/c or C57BL/10 chromosome. Therefore, as shown in Figs. 3 and 4, the V_H3609 family of CXXB F2 and F52 is presented as a single, half-filled box to indicate the lack of assignment to a particular allele.

The Most 5′ V_H Gene Segments are Members of the V_HJ558 Family. The great complexity of the V_HJ558 family has impeded the construction of a fine-resolution map of these genes. CXXB F102 has no detectable V_H gene sequences except for five V_HJ558 Eco RI fragments, indicating that these genes are the most 5′ V_H sequences we have identified. CXXB F52 and F2, each of which has deleted most germline V_H3609 genes, have retained 13 and 16 V_HJ558 Eco RI fragments, respectively. Several other cell lines (CXXB F29, M2, M38) have clearly deleted some V_HJ558 members. However, the large number of V_HJ558 fragments (~35) makes it difficult to detect a partial loss of this family. Indeed, it is possible that most V_HJ558 hybridizing Southern blot bands represent a number of unique, comigrating fragments that may not map in the same region of the *Igh* locus.

Evidence that a V_HJ558 Family Member Maps 3′ of V_HX24. A-MuLV-transformed lines were generated from (BALB/c × C.AL-20)F_1 mice (Igh^a × Igh^c). One of these cell lines, CXAL 1.40, has deleted V_HS107 (V3-V1), V_HQ52, and V_H7183 from the BALB/c chromosome (Fig. 4). We inferred that CXAL 1.40 has rearranged a V_H sequence that is located between V_HX24 and V_HS107 (V3-V1) in the BALB/c germline. To determine the nature of this rearranged sequence, we cloned both J_H rearrangements. One cloned fragment hybridized with a V_HJ606 probe and, based on the deletion profile of CXAL 1.40 (Fig. 4), most likely contains the VDJ rearrangement of the C.AL-20 (Igh^c) chromosome. DNA sequencing of the other V_HDJ_H rearrangement, however, revealed a V_H gene with identity to the germline V_H gene H4a-3, which belongs to the V_HJ558 family. The H4a-3 gene is one of a small number of BALB/c germline genes recognized, under high stringency, by a cDNA V_H probe derived from the anti-GAT mAb, G8 Ca 1.7 (30). Although not yet formally proven, based on the deletion profile of CXAL 1.40 (Fig. 4) and the sequence identity with a BALB/c germline V_H gene, we propose that the cell line rearranged a V_HJ558 gene on the BALB/c chromosome. Furthermore, using *Igh*-recombinant strains, at least one V_H gene with high homology to our CXAL 1.40 sequence has been mapped to a region that includes the V_HS107 V11 gene and part of the V_HS107 V11 to V3 interval (Tutter, A., P. Brodeur, M. Shlomchik, and R. Riblet, submitted for publication). We provisionally conclude, therefore, that at least one V_HJ558 member is located between V_HX24 and V_HS107 (V3-V1) in the Igh^a haplotype.

We have examined the CXAL 1.40 cell line for the presence of V_HARS (V_H36-65), the V_HJ558 member (1) expressed in the predominant idiotype response (CRI) to *p*-phenyl-arsonate in Igh^c strains (31). As previously reported, the V_H36-65 probe, VId-130 (32), hybridizes to the V_HARS-bearing Eco RI fragment (6.2 kbp) of the Igh^c haplotype but does not hybridize to BALB/c (Igh^a) genomic DNA. In agreement with the data of Rathbun et al. (12), we have demonstrated that the V_HARS gene is retained by CXAL 1.40 (Fig. 6), indicating that this gene is 5′ of the rearranged V_HJ606 gene on the Igh^c chromosome. The Southern blot shown in Fig. 6 was also hybridized with a β_2-microglobulin probe (23). The comparable intensities of the 1.8-kbp β_2 fragment verify that each lane contained approximately the same amount of DNA.

The Relative Chromosomal Position of Nine V_H Gene Families can be Inferred from Deletional Analysis. The data described above allow the positioning of 13 "clusters" of V_H genes. Fig. 7 shows the relative order of these clusters along the chromosome and indicates the interspersion (V_HQ52, V_H7183) and dispersion (V_HS107, V_H36-60, VGAM3.8) of the family clusters.

Discussion

We have constructed a panel of pre-B cell lines to elucidate the organization of the *Igh* locus by deletion mapping. We have determined the positions of nine V_H gene families relative to the D-J_H-C_H region on chromosome 12 of BALB/c and C57BL/10 mouse strains. The members of a given V_H gene family are generally clustered within a limited region of the locus, although three families consist of two clusters separated by members of one or more different families. The inferred map order is 5′-V_HJ558-V_H3609-V_HJ606-V_H36-60-VGAM3.8-V_H36-60-V_HS107(V13-V11)-VGAM3.8-V_H36-60-V_HX24-V_HS107(V3-V1)-V_HQ52/V_H7183-D-J_H-C_H-3′. As sug-

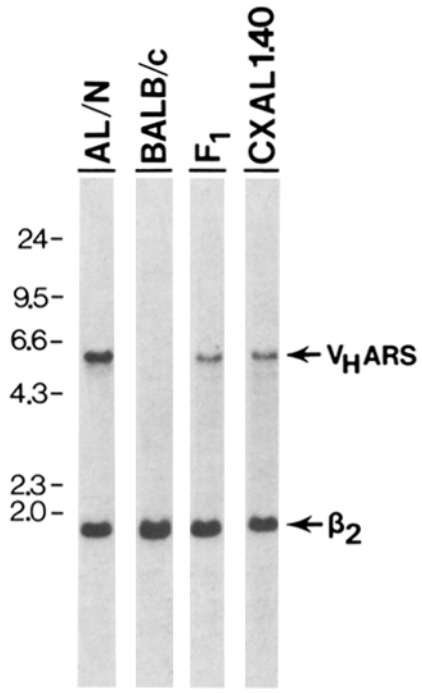

FIGURE 6. The V_HARS (V_H36-65) gene is 5′ of the V_HJ606 family. DNAs from the CXAL 1.40 cell line and livers of AL/N, BALB/c, and (BALB × AL)F_1 mice were digested with Eco RI, transferred to Nytran membrane, and hybridized with a mixture of VId130 (V_HARS) probe (31) and β_2-microglobulin probe (23).

gested by previous studies (11, 12, 25, 26), the two most D-proximal V_H families are completely interspersed and the two families most distal to the D region, V_HJ558 and V_H3609, may be partially interspersed.

We initially reasoned that a valid V_H gene map based on deletion analyses would require that deletions on all chromosomes of the same haplotype be consistent with a single map order. Such a map should be based on many independent deletions, yielding multiple consistent orderings. Our panel consists of 51 independently rearranged *Igh-V* loci, all of which have deletion profiles consistent with a single V_H map (Figs. 3, 4). We believe, therefore, that the size and consistency of the database presented here provide compelling support for the order of V_H gene families shown in Fig. 7.

Recent studies using deletion mapping have been reported by Rathbun et al. (12) and Blankenstein and Krawinkel (11). Both studies examined a limited set of "haploid" cell lines. Rathbun and colleagues examined five cell lines and suggested a map order of V_H3609-V_HJ558-(V_HJ606, VGAM3.8, VHS107)-V_H36-60-(V_HX24, V_HQ52, V_H7183). Blankenstein and Krawinkel examined two haploid hybridomas, one Igh^a and one Igh^b, and a single recombination to infer a relative map order that differs significantly from that of Rathbun et al. (12) and the map derived from the present study. We do not know the causes of the discrepancies in map orders inferred from deletion analyses of hybridoma cell lines and that inferred from our data. It is possible that the inherent instability and complexity of hybridoma genomes (aneupoidy, translocations) and the possible anomolies associated with haploid loci generated in tissue culture lines could explain the differences. In contrast, the large number

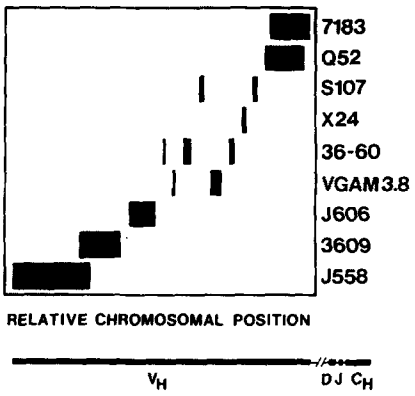

FIGURE 7. Relative chromosomal position of nine V_H gene families. The "complexity" of each family cluster is indicated by the lengths of the solid bars (V_HX24 = 2). Actual physical distances are unknown.

of independent data points (51 deletions), the inherent stability of A-MuLV-transformed lines (33), and the internal consistency of our data strongly support the V_H gene map inferred from our study.

V_H gene deletion analyses of rearranged chromosomes of both *Igh*^{a} and *Igh*^{b} haplotypes were consistent with a single V_H gene family organization. More limited data for the *Igh*^{n}, *Igh*^{e}, and *Igh*^{j} haplotypes are also consistent with the V_H gene chromosomal positions shown in Fig. 7 (Fig. 4 and unpublished results).

Differences in V_H family utilization between BALB/c and C57BL strains have been reported (5, 7), although such differences have not been consistently observed in all systems (6). Based on our finding that V_H gene organizations of *Igh*^{a} and *Igh*^{b} haplotypes are indistinguishable, it is unlikely that differences in family utilization are due to significant differences in the relative map positions of the V_H gene families. Indeed, Wu and Paige (34) and Jeong et al. (7) have presented evidence that such differences are controlled by other (non-*Igh*) loci.

The suggestion that mouse V_H genes belonging to the same family are clustered was first made by Kemp et al. (35) after isolating distinct genomic DNA clones, each bearing pairs of highly related V_H sequences (V_HS107 and V_HJ606). Bothwell et al. (36) and Givol et al. (37) reported similar findings for members of the V_HJ558 family. Previous analyses of B cell lines for V_H gene deletions (11, 12, 38, 39) were consistent with a clustered organization of V_H gene family members. V_H gene analysis of *Igh*-recombinant mouse strains constructed by Riblet (40) also indicated a generally clustered organization of V_H families (10).

The clustered nature of mouse V_H gene families contrasts with the organization of the human V_H locus, which appears to have a more interspersed distribution of family members (41, 42). Although the data are currently more limited, the mouse Vκ genes also appear to have generally clustered family members (43, 44) while the human Vκ genes are highly interspersed (45). Data from wild-mouse populations and other mammals are not yet available.

The high degree of complexity and relative map position of the V_HJ558 family impose certain limits on the present analysis. Because this set of V_H genes consists of at least 60 (1) and perhaps 500 (46), it is difficult to resolve very many polymorphic fragments on Southern blots. Furthermore, the fact that V_HJ558 members map most distal to the D region makes it difficult to ascertain whether any V_HJ558 genes

are dispersed within the more D-proximal families. However, based on our finding that the cell line CXXB M18 has deleted all members of V_HJ606, VGAM3.8, V_H36-60, V_HS107, V_HX24, V_HQ52, and V_H7183 but has retained an apparently complete *Igh*^{b} V_HJ558 pattern, we suggest that most V_HJ558 members are 5′ of V_HJ606. Nevertheless, the apparent location of a V_HJ558-like gene between V_HX24 and V_HS107 (V3-V1) on the BALB/c chromosome in the CXAL 1.40 cell line is supported by the finding that a gene of the H10/Sm7 (30, 47) set of V_HJ558-related sequences maps to a distinct region 3′ of the major V_HJ558 cluster (Tutter, A., P. H. Brodeur, M. Shlomchik, and R. Riblet, submitted for publication). The finding of V_HJ558 members in this 3′ region has important implications. First, it reveals that V_HJ558-related genes are dispersed over a wide range in the *Igh-V* locus. Second, it underscores the importance of using hybridization probes derived from distinct members of the V_HJ558 family, under high stringency conditions, to operationally define V_HJ558 family subsets and to apply deletion mapping analysis to map such subsets. With these means, a significant resolution of this complex set of genes may be attained. Third, the location of the H10/Sm7-like genes in the region between V_HX24 and V_HS107 (V3-V1) has implications for the origins of V_H family dispersion and for the evolutionary history of the *Igh-V* locus as discussed below.

There are three major groups of mouse V_H genes (I, II, III) based on the original classification of Kabat et al. (48) as recently modified by Tutter and Riblet (49). Accordingly, members of V_HQ52, V_H36-60, and V_H3609 belong to group I and are more closely related, by sequence, to each other than to other V_H gene families. As shown in Fig. 7, these three families are located in distinct regions of the *Igh* locus and are not adjacent. Similarly, family clusters of group II (VGAM3.8, V_HJ558) and those of the group III families (V_H7183, V_HS107, V_HX24, V_HJ606) are generally separated by members of one or two different groups (Fig. 8). This organization suggests a possible evolutionary pathway in which divergence of the ancestral groups II, I, and III (in that order) was followed by two independent duplications of the locus resulting in four repeats of the II-I-III unit. V_H families may have then evolved by more recent divergence including, perhaps, expansions and contractions. There is, in fact, evidence for both a large duplication within the human IgVκ locus (50) and for smaller, incremental changes within mouse V_H gene families (51).

Several distinct mechanisms have been invoked to account for the evolution of multigene families (52). The interspersion of V_HQ52 and V_H7183 family members, typical of the human V_H (41) and Vκ loci as well as both mouse and human T cell receptor V_β genes (53, 54), may be the product of illegitimate recombination be-

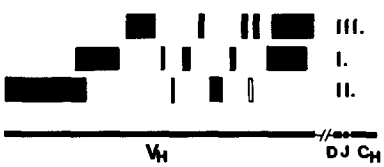

FIGURE 8. Relative chromosomal position of the three major V_H groups (I, II, III) based on nucleotide sequence similarities (49): Group I, V_HQ52, V_H3609; Group II, VGAM3.8, VH36-60, VHJ558; Group III, VH7183, VHS107, VHX24, VHJ606. Solid rectangles indicate the relative chromosomal position of VH gene clusters. The length of each rectangle indicate the approximate number of V_H gene-bearing restriction fragments within a given cluster. The position of the D-proximal Group II cluster is provisional (see Results), and is, accordingly, illustrated by an open rectangle.

tween sequences (repetitive or nonrepetitive) conserved throughout a particular locus. Alternatively, the creation, expansion, and conservation of VH gene clusters might occur via homologous, unequal recombination and/or gene conversion events. It is possible that the only selective pressure on V region genes is the maintenance of a minimal V gene pool and the *Igh-V* locus may be permissive to a wide variety of evolutionary changes in size, composition, or organization. On the other hand, certain, as yet unrecognized, features of V gene organization may be important in the development of the antigen specific receptor repertoire, for example, the developmental read-out of particular V genes (55, 56) or antigen specificities (57, 58).

Despite the apparent evolution of a highly diversified immune system, various studies have demonstrated a biased usage of V_H gene segments most proximal to the D_H segments (V_H7183 family) in pre-B cells. Yancopoulos et al. (55) examined 9 A-MuLV-transformed BALB/c fetal liver derived pre-B cell lines, and documented 12 VDJ_H rearrangements in these cell lines, of which 11 involved the V_H7183 gene family. Similar results were obtained by Perlmutter et al. (56), who found that 7 of 9 fetal liver hybridomas express the VH7183 family. We have recently generated fetal liver A-MuLV pre-B cell lines to examine the V_H gene usage during ontogeny in CBA/Tufts mice. In contrast to initial reports, we and others (59-61) have observed the preferential utilization of both V_H7183 and V_HQ52 family members in pre-B cells (62). Taken together, the findings by ourselves and others (25, 26, 59) that V_H7183 and V_HQ52 family members are interspersed in a variety of haplotypes, and that both families are preferentially utilized in pre-B cells, strongly support the suggestion of Yancopoulos et al. (55) of a position-dependent rearrangement of V_H gene segments in early ontogeny.

That the number and position of functional genes within a given family are unknown complicates attempts to relate V_H gene family representation in the adult repertoire to gene organization. Thus, the relative contributions of family complexity (1) and chromosomal position as determinants of V_H gene representation in the adult primary B cell repertoire are not clear. However, independent analyses have suggested that the most D-distal V_H gene families (V_HJ558 and V_H3609) are underrepresented relative to their apparent complexities. For example, the V_HJ558 family is utilized only about six times more frequently than the three functional genes of the V_HS107 family in BALB/c adult splenic B cell populations (7, 63, Sheehan, K. M., and P. H. Brodeur, manuscript in preparation). If all functional V_H segments have an equal probability of rearrangement and expression (as with a simple complexity driven model), the calculated number of functional V_HJ558 family genes would be approximately eighteen (3 × 6). An estimate of 18 functional V_HJ558 genes requires either a very large proportion of dysfunctional genes in this family, for which size estimates range from a minimum of 60 (1) to >500 members (46), or other factors that influence V_H gene utilization. The uncertainty of the actual number of functional V_HJ558 genes and the demonstration of non-*Igh* locus influence on V_H gene usage (34), limits the impact of the data cited above in supporting a strict position-dependent model of V_H gene expression. Therefore, additional analyses will be required to ascertain the full extent of and the basis for the shift from preferential rearrangement of D-proximal V_H genes early in ontogeny (55, 63), toward a more probabilistic pattern of V_H gene utilization that occurs in adult lymphoid tissues (7, 63).

Summary

We have constructed a panel of Abelson murine leukemia virus-transformed pre-B cells to study the organization of the mouse V_H gene families. Based on the analyses of V_H gene deletions on 51 chromosomes with V_H gene rearrangements, we have inferred a map order of the *Igh* locus that holds for both the Igh^a and Igh^b haplotypes.

We show that members of each V_H gene family are generally clustered, although three family clusters (V_HS107, V_H36-60, VGAM3.8) are dispersed in two or three subregions of the locus. Members of two V_H gene families, V_HQ52 and V_H7183, are extensively interspersed and map within the same subregion. An examination of the distribution of V_H group members (V_H II, I, and III) within the locus suggests that two major duplications may, in part, explain the dispersed pattern of V_H family clusters. The relationship of V_H organization and functional expression is discussed in terms of position-dependent and complexity-driven models.

The authors wish to dedicate this paper with affection and respect to the memory of Dr. Frances L. Owen, our colleague, teacher, and friend, in recognition of her outstanding contribution to immunology during her deeply committed but unfortunately short life.

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