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Chromosomal organization of the heavy chain variable region gene segments comprising the human fetal antibody repertoire

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ABSTRACT **The adult repertoire of antibody specificities is acquired in a developmentally programed fashion that, in mouse and man, parallels the ordered rearrangement of a limited number of germ-line heavy chain variable region (V_H) gene segments during development. It has been hypothesized that this developmental bias is a consequence of gene organization. In the mouse, rearrangement of V_H gene segments proximal to the heavy chain joining region (J_H) locus precedes rearrangement of genes located more distal to the J_H locus. Similarly, in man, two V_H elements located proximal to J_H are expressed during fetal development. To test further this hypothesis in man, we have determined in a single individual the positions of an additional eight distinct V_H elements known to comprise a significant fraction of the human developmental repertoire. These developmentally expressed V_H elements were found to be dispersed over a region of 890 kilobases of the V_H locus and were interspersed with other V_H elements that are not known to be developmentally expressed. Thus, the ordered developmental expression of V_H gene segments in man must involve mechanisms beyond physical proximity to the J_H locus. Further, these results support the notion that fetal expression of V_H gene segments is a regulated process and suggest that this regulation is important in the acquisition of immunocompetence.**

Certain heavy chain variable region (V_H) elements are preferentially expressed during fetal development (1–11). This preference is thought to result from constraints on V_H rearrangement during development (3–5). One hypothesis is that the principal factor guiding selection of the fetally expressed repertoire is chromosomal location (3–5). In support of this notion, the single member of the human V_H6 gene family has been unambiguously mapped to the most 3′ region of the V_H locus (12, 13) and has been identified as the germ-line source of the developmentally expressed V_H element 15p1 (4, 10). Furthermore, cloning and mapping have revealed that on at least one haplotype the first element 5′ to V_H6 encodes the fetally expressed V_H1 gene segment 20p3 (4, 14). An additional restricted subset of genes from the V_H1, V_H3, and V_H4 gene families has been found to be fetally expressed (4, 10, 11), but it is unknown to what extent their fetal expression is a function of proximity to the heavy chain joining region (J_H).

A physical map of the V_H locus has established the general location of 76 restriction fragments carrying members of the known V_H families (15, 16). More recently, we have determined the chromosomal order of the set of *Bgl* II restriction fragments carrying members of the V_H4 gene family (17). This was accomplished by analysis of the pattern of loss of hybridizing bands from the genomes of a panel of monoclonal lymphoblastoid cell lines (LCLs) that had well-characterized rearrangements of the immunoglobulin locus on each haplotype (17). Physical dimensions were supplied to this genetic map by the assignment of these ordered elements to large [50–350 kilobases (kb)] restriction fragments using two-dimensional pulsed-field gel electrophoresis (PFGE) (17). Six fragments spanning 890 kb were ordered.

We now report the mapping of V_H elements known to be expressed during fetal development using the previously established V_H locus map. The results demonstrate that the developmentally expressed V_H gene segments studied are dispersed throughout the V_H locus and are interspersed with V_H gene segments that are not known to be developmentally expressed, indicating that expression of a subset of V_H gene segments early in development is regulated by factors other than physical proximity of the diversity or joining region gene segments.

MATERIALS AND METHODS

V_H Gene-Specific Probes. The use of oligonucleotide probes to detect V_H gene segments in genomic DNA and the sequences of the probes H110 (18), M19, H61, M16 (19), and H37 (20) have been described previously. Additional probes and target genes [codon numbering according to Kabat *et al.* (21)] are as follows: M8, 5′-AGCAGCTATGCCATGAGCTGG-3′, codons 30–36, 30p1 (4); M24, 5′-GGTGGTAGCACATACTACGCA-3′, codons 54–60, 30p1; M85, 5′-AGTAGCTACGACATGCACTGG-3′, codons 30–36, 38p1 (4); M52, 5′-GCTGGTGACACATACTATCCA-3′, codons 54–60, 38p1; H111, 5′-GGGATCATCCCTATCTTTGGT-3′, codons 50–55, 51p1 (4); M27, 5′-TTTGGTACAGCAAACTACGCA-3′, codons 54–60, 51p1; M44, 5′-AGGATCATCCCTATCCTTGGT-3′, codons 50–55, hv1263 (22); M41, 5′-GGAAGTAATAAATACTACGCA-3′, codons 54–60, hv3005 (23); M76, 5′-GCAGTTATATGGTATGATGGA-3′, codons 49–54, hv3019b9 (24); E7, 5′-TCCATCAGTAGTTACTACTGG-3′, codons 28–34, 58p2 (4).

Germ-Line Genes. A genomic library constructed by cloning DNA derived from peripheral blood lymphocytes of donor 3116 in the phage λEMBL3 vector was screened with the probes described in Table 1. The V_H gene segments in plaque-purified clones were PCR amplified for 30 cycles (1 min, 94°C; 2 min, 55°C; 3 min, 70°C) using the following V_H gene family-specific primers: V_H1, 5′-TTCTTGGTGGCAGCAGCCACAGG-3′, and 5′-AGGATGTGGTTTCTCACACTGTG-3′; V_H3, 5′-TGTTTGCAGGTGTCCAGTGT-3′ and 5′-CAATGACTTCCCCTCACTGTG-3′; V_H4, 5′-CCTCTAGACCCAGGGCTCACTGTGGG-3′ and 5′-CCCGGGCTCACACTCACCTCCCCT-3′. PCR products were cloned into the *Hin*cII site of M13mp19. Nucleotide sequences of the inserts were determined by the dideoxynucleotide chain-

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Abbreviations: J_H, heavy chain joining region; V_H, heavy chain variable region; PFGE, pulsed-field gel electrophoresis; LCL, lymphoblastoid cell line; CDR, complementarity-determining region.

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termination method using the Sequenase V2.0 kit (United States Biochemical).

Image Analysis. The amount of hybridization of bands was determined by storage phosphor imaging (25) (using a Molecular Dynamics 400A PhosphorImager provided by Paul C. Goodwin, Image Analysis Laboratory, Fred Hutchinson Cancer Research Center, Seattle, WA) and was corrected for the amount of DNA loaded by normalization against the amount of hybridization of known single-copy probes. Probes used for normalization were oligonucleotide M49, 5′-AAGGATAAAAATGAAGATGGA-3′, a chromosome 14-specific probe that detects two alleles of the T-cell receptor Vα-21 gene segments in *Taq* I-digested DNA (E.C.B.M., C. M. Alexander, J. Wright, and P. Concannon, unpublished observation), and oligonucleotide H30, 5′-GCCGTGTCCTCGGCTCTCAGG-3′, which detects a chromosome 4 variable number of tandem repeats (D4S139) (26). Both probes gave similar results for normalization.

RESULTS

Identification of Germ-Line Counterparts of Developmentally Expressed V_H Gene Segments. We and others have shown previously that oligonucleotide probes, derived from known germ-line V_H sequences, can be employed to assign these V_H gene segments to specific restriction fragments (18, 19, 27, 28). To identify the germ-line restriction fragments carrying developmentally expressed V_H gene segments, we have developed a panel of oligonucleotide probes that detect these and related elements specifically (Table 1). Corresponding germ-line genes were obtained from a genomic recombinant phage library by screening with this same set of probes. The germ-line V_H gene segments carried on the isolated phage clones were PCR amplified and sequenced. For seven of the eight developmentally expressed cDNA sequences analyzed, identical germ-line V_H gene segments were obtained (Table 1).

V_H clone 3d23 is identical to the 20p1 sequence and is identified uniquely by probe H110 on a 3.1-kb *Taq* I fragment (18) (Fig. 1).

V_H clone 3d131 is identical to the 30p1 sequence. Probe M8 identifies possible germ-line sources of this element on 7.0- and 5.0-kb *Taq* I fragments (Fig. 1). A panel of nine oligonucleotide probes covering >50% of the 30p1 sequence consistently hybridized to these restriction fragments (data not shown). Thus, based on oligonucleotide probe hybridization analysis, it was not possible to distinguish between the elements carried on the 7.0- and 5.0-kb *Taq* I fragments. The V_H gene segments on the 7.0- and 5.0-kb *Taq* I fragments detected by probe M8 are presumably identical to the 30p1 sequence and were designated germ-line 30p1A and 30p1B, respectively.

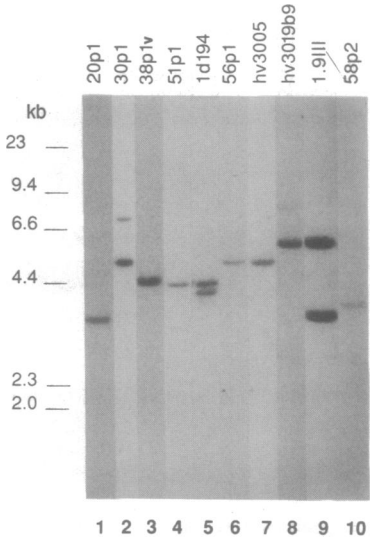

FIG. 1. Sequence-specific identification of individual V_H gene segments. Genomic DNA derived from donor 3116 was digested with *Taq* I (lanes 1–9) or *Bgl* II (lane 10) and hybridized to oligonucleotide probes specific for the V_H elements indicated. Probe name, hybridization and washing temperatures, and film exposure times are as follows: Lane 1, H110, 57°C, 57°C, 3 days; lane 2, M8, 53°C, 9 days; lane 3, M85, 50°C, 56°C, 7 days; lane 4, H111, 53°C, 53°C, 5 days; lane 5, M27, 56°C, 56°C, 5 days; lane 6, M19, 54°C, 59°C, 10 days; lane 7, M41, 47°C, 59°C, 10 days; lane 8, M76, 49°C, 59°C, 6 days; lane 9, M16, 52°C, 60°C, 6 days; lane 10, E7, 53°C, 54°C, 12 days.

The 38p1 complementarity-determining region 2 (CDR2) sequence is identified uniquely by probe M85 on a 4.4-kb *Taq* I fragment (Fig. 1). V_H clones 3d136 and 3d140 are highly homologous to 38p1 and are identical to 38p1 throughout CDR2. The V_H clone 3d136 differs from 38p1 by one nucleotide, and V_H clone 3d140 differs by three nucleotides (data not shown). The V_H clones 3d136 and 3d140 are presumed to be allelic variants of 38p1 and are indicated as 38p1v.

The 51p1 sequence is represented by phage clone 1d144 and is identified by probe H111 on a 4.3-kb *Taq* I fragment (Fig. 1). Probe M27, which is derived from a different region of the 51p1 cDNA sequence, identifies this 4.3-kb *Taq* I fragment as well as a 4.0-kb *Taq* I fragment (Fig. 1). The 4.0-kb *Taq* I fragment carries a 51p1-related V_H gene segment, differing from the 51p1 sequence in the region of CDR2 covered by probe H111. This second V_H1 gene segment has been isolated as V_H clone 1d194. The 1d194 sequence is 98% (288/294) identical to the 51p1 sequence (data not shown).

Table 1. Characteristics of germ-line V_H gene segments of donor 3116

V_H gene segment	Fetal expression*	V_H family	Diagnostic probes	Fragment size,† kb	Phage clones
51p1	+	V_H1	H111, M27	4.3	1d144
1d194	−	V_H1	M27, M44	4.0	1d194
20p1	+	V_H3	H110, M22	3.1	3d23
30p1A	+	V_H3	M8, M24	7.0	3d131
30p1B	+	V_H3	M8, M24	5.0	3d131
38p1v	−	V_H3	M85, M52	4.4	3d136, 3d140
56p1	+	V_H3	M19, H61	5.0	3d216
hv3005	−	V_H3	M41, H61	5.0	3d24
hv3019b9	+	V_H3	M76, M16	6.0	3d277
1.9III	+	V_H3	M16, H61	3.5	3d28
58p2	+	V_H4	E7, H37	3.6	3d197d

*+, Exact sequence reported by Schroeder *et al.* (4, 10) or by Raaphorst *et al.* (11); −, related elements that differ by 1 or more base pairs from fetally expressed sequences reported by Schroeder *et al.* (4, 10) or by Raaphorst *et al.* (11).

†Restriction enzyme is *Taq* I for all elements except 58p2, which is *Bgl* II.

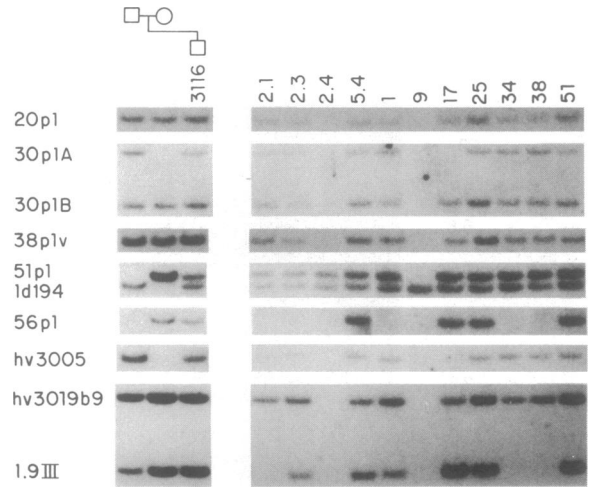

FIG. 2. Deletional analysis of individual V_H gene segments. Genomic DNA derived from Virginia Mason Research Center family 117, which includes donor 3116, and genomic DNA derived from the LCLs was digested with *Taq* I and hybridized to oligonucleotide probes diagnostic for the V_H gene segments indicated. V_H gene segments and diagnostic probes are as follows: 20p1, H110; 30p1A and 30p1B, M8; 38p1v, M85; 51p1 and 1d194, M27; 56p1, M19; hv3005, M41; 1.9III and hv3019b9, M16. Hybridization conditions were as in Fig. 1.

V_H clone 3d216 is identical to the 56p1 sequence and is identified on a 5.0-kb *Taq* I fragment by probe M19 (Fig. 1) (19). V_H clone 3d24 is identical to the gene hv3005 (23) and is also identified on a 5.0-kb *Taq* I fragment by probe M41 (Fig. 1) (19). Thus, germ-line 56p1 and hv3005 comigrate in *Taq* I digests and have been postulated to be alleles (19). Oligonucleotide probe M16 is specific for hv3019b9 (24) and 1.9III (13) and detects fragments of 6.0 and 3.5 kb (Fig. 1). V_H clone 3d277 is identical to V_H element hv3019b9 and is specifically identified by probe M76 on the 6.0-kb *Taq* I fragment (Fig. 1). V_H clone 3d28 is identical to V_H element 1.9III and is identified by the unique combination of M16 and H61 on the 3.5-kb *Taq* I fragment (19).

In previous work, we have localized the 58p2 sequence to either or both 3.7- and 3.6-kb restriction fragments in *Bgl* II-digested germ-line DNA (referred to as V_H4 bands 11 and 12, respectively) (17, 27). V_H clone 3d197d is identical to 58p2 and, as shown in Fig. 1, is specifically identified by probe E7 on the 3.7-kb *Bgl* II fragment (band 12 in ref. 17).

Deletional Analysis of Developmentally Expressed Germ-Line V_H Gene Segments. A previous study of a panel of chromosome 14-specific deletion lines (17) provided a detailed order of V_H4 gene segments in the V_H locus of a single donor and an estimate of the physical region spanned by the V_H locus. Using these deletion lines, it is possible to assign chromosomal locations of V_H gene segments by hybridization analysis.

The results of sequence-specific hybridization to DNA derived from the LCLs, using one diagnostic oligonucleotide probe per V_H gene segment, are shown in Fig. 2. Also shown are the hybridization patterns of germ-line DNA derived from family 117, which includes donor 3116, enabling the assignment of specific V_H gene segments to the paternal chromosome, the maternal chromosome, or both. A number of bands segregate unambiguously. Thus, the 7.0-kb *Taq* I band detected by probe M8 and designated germ-line 30p1A can be assigned to the paternal chromosome. The 4.3-kb *Taq* I band detected by H111 (51p1) and the 5.0-kb *Taq* I band detected by M41 (hv3005) can also be assigned to the paternal chromosome. Similarly, the 4.0-kb *Taq* I band detected by M27 (1d194) and the 5.0-kb *Taq* I band detected by M19 (56p1) can be assigned to the maternal chromosome (Fig. 2). The hybridization patterns of the haplotype-specific elements were scored by inspection (Table 2). The amount of hybridization of the bands that did not segregate unambiguously (bands carrying 20p1, 30p1B, 38p1V, hv3019b9, and 1.9III sequences) was determined using quantitative phosphor image analysis. A band intensity ratio (band intensity in the LCL/band intensity in 3116 germ line) was calculated from the normalized image data. This ratio was used to estimate the number of copies of these V_H gene segments in the bands (Table 2).

A rough order of V_H gene segments was determined from the frequency with which each element was deleted (Table 2). Final ordering of the elements was achieved by careful analysis of haplotype-specific deletions of the elements described in this study and comparison with the haplotype-specific deletion patterns of the V_H4 bands described previously (17). These analyses revealed that the nonsegregating elements 20p1, 30p1B, 38p1V, and hv3019b9 displayed deletion patterns consistent with their presence on both parental chromosomes. This is demonstrated by the hybridization

Table 2. Hybridization patterns of V_H gene segments in the LCLs

	V_H gene segment (copy number)											
Line	1d194	51p1	58p2	hv3019b9	1.9III	56p1	hv3005	30p1A	30p1B	38p1v	20p1	15p1
L2.1	1	1	2	1	0	0	1	1	1	1	1	1
L38	1	1	2	1	0	0	1	1	1	1	1	1
L2.3	1	1	2	2	1	0	1	1	1	1	1	1
L1	1	1	2	2	1	1*	1	1	1	1	1	1
L17	1	1	2	1	2	1	0	0	1	1	1	1
L5.4	1	1	2	2	2	1	1	1	2	2	2	1
L25	1	1	2	2	2	1	1	1	2	2	2	1
L9	1	0	1	0	0	0	0	0	0	0	0	0
L2.4	1	1	2	0	0	0	0	0	0	0	0	0
L34	1	1	1	1	0	0	1	1	1	1	1	0
L51	1	1	2	2	2	1	1	1	2	2	2	1
G.L.†	1	1	2	2	2	1	1	1	2	2	2	2
% del‡	0	9	9	36	55	55	27	27	45	45	45	68

Data were obtained from quantitation of the hybridizations shown in Fig. 2 using phosphor image analysis. Data for 15p1 and 58p2 have been reported previously (17).

*Data for the 56p1 sequence in LCL L1 were obtained with probe H61. As explained in the text, LCL L1 is blank for M19 (Fig. 2) because of a somatic mutation.

†Germ-line DNA derived from donor 3116 from whom all LCLs were derived.

‡The frequency of deletion of a particular V_H element is shown as a percentage of the number of relevant chromosomes 14 analyzed.

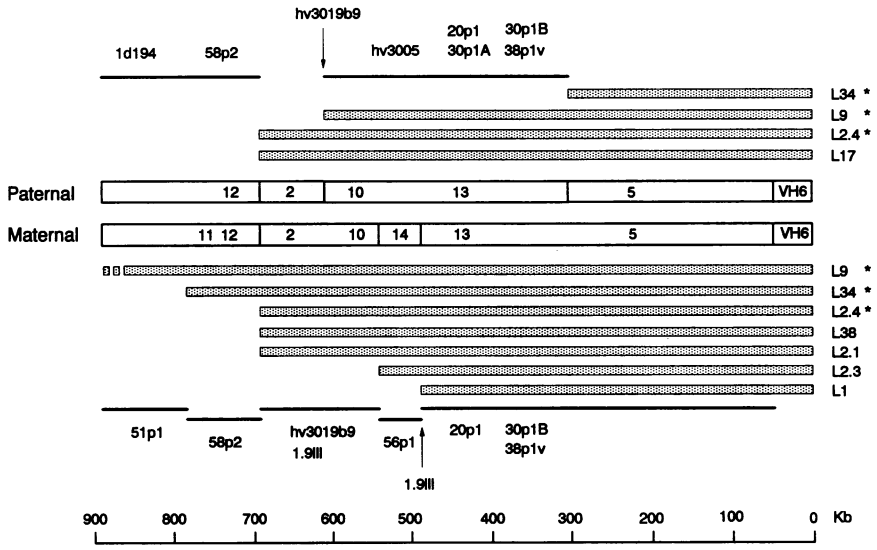

FIG. 3. Map of the V_H locus in donor 3116. The order and presence of V_H4 elements on the parental chromosomes are shown in the open boxes. The extent of deletions on the paternally derived chromosome is indicated above the map and the extent of deletions on the maternally derived chromosome is indicated below the map. An asterisk (*) indicates LCLs that have V_HDJ_H (D indicates diversity) rearrangements on both chromosomes. The elements mapped in this study are indicated above and under horizontal bars, which show the maximum extent of the region to which these elements map. V_H4 band 12 has been identified as carrying the 58p2 sequence and both identifications are indicated. Arrows indicate rearrangement of the hv3019b9 gene segment on the paternal chromosome in L9 and of the 1.9III gene segment on the maternal chromosome of L1 (data not shown). The indicated physical dimensions of the map and extent of deletions in the LCLs are approximate. [Reproduced (with modification) with permission from ref. 17 (copyright Journal of Immunology).]

results of the three LCLs that have extensive V_H locus deletions on one parental chromosome and an intact V_H locus on the other chromosome. LCLs L2.1 and L38 have each deleted ≈700 kb of the V_H locus on the maternal chromosome, while retaining an intact V_H locus on the paternal chromosome. Similarly, LCL L17 has deleted ≈700 kb of the V_H locus on the paternal chromosome, while the V_H locus on the maternal chromosome is intact (17). The bands carrying 20p1, 30p1B, 38p1V, and hv3019b9 are all half intensity in LCLs L2.1, L38, and L17, consistent with their presence on both parental chromosomes (Table 2). In contrast, the band carrying 1.9III is full intensity in LCL L17 but absent in LCLs L2.1 and L38. This assigns one or more copies of the 1.9III sequence exclusively to the maternal chromosome. Since the band carrying 1.9III in LCLs L2.3 and L1 is half intensity (both lines have deletions of the V_H locus on the maternal chromosome only), 1.9III must be duplicated on the maternal chromosome. Also, since the band carrying the 30p1B sequence is apparently present on both parental chromosomes, the bands carrying the 30p1A and 30p1B sequences must represent two separate loci. A map of the haplotypes, including the developmentally expressed V_H gene segments and the previously mapped V_H4 *Bgl* II bands, is shown in Fig. 3. Fig. 3 also shows the extent of the deletions in the LCLs as estimated previously by one- and two-dimensional PFGE (17).

Somatic Mutation of Unrearranged V_H Gene Segments. To minimize false negatives due to somatic mutation, at least two independent oligonucleotide probes were used for every V_H gene segment mapped in this study (Table 1 and data not shown). Hybridization results obtained with different probes that detected the same element were identical in all instances except for one, which was the 56p1 sequence in LCL L1. Donor 3116 possesses the 56p1 and hv3005 sequences as indicated by hybridization to the sequence-specific probes M19 and M41, respectively (Fig. 1). Probe H61 does not distinguish between the 56p1 and the hv3005 sequences under commonly used stringency conditions (19). Since the intensity of the 5.0-kb *Taq* I band detected by probe H61 in donor 3116 and LCL L1 was identical (data not shown), it was inferred that neither the 56p1 sequence nor the hv3005 sequence is deleted from LCL L1. Therefore, the failure of the 56p1-specific probe, M19, to hybridize to LCL L1 (Fig. 2) indicates the presence of a somatic mutation in the 56p1 sequence in LCL L1. The presence of a single C → T transition in the third position of codon 58 (21) was confirmed by cloning and sequencing the unrearranged 56p1 sequence from this LCL.

DISCUSSION

It has been thought that chromosomal location proximal to J_H determines preferential expression of a subset of V_H gene segments early in development (3, 5). Indeed, two developmentally expressed V_H gene segments map to the extreme 3′ end of the human V_H locus (12–14). In this study, an additional eight developmentally expressed V_H gene segments were positioned in the V_H locus and were found to be distributed over 890 kb of the V_H locus. Two V_H elements, encoding the developmentally expressed 51p1 and 58p2 sequences, map at least 690 kb 5′ of V_H6, the most 3′ element in the human V_H locus. The hv3019b9 gene segment, which encodes the developmentally expressed sequences FL13-2 and FL13-48 (11), is located ≈600 kb 5′ to V_H6. The 1.9III gene segment, which encodes the developmentally expressed sequence, FL13-42, is duplicated; one copy is located in the interval between 540 and 690 kb 5′ to V_H6, and the second copy is located ≈490 kb 5′ to V_H6. The element encoding the 56p1 sequence, which was the most frequently expressed element at days 104 and 130 of fetal development (4, 10), maps at least 450 kb 5′ of V_H6. Germ-line 20p1, 30p1A, 30p1B, and 38p1v map to the 450-kb region immediately upstream of V_H6 (Fig. 3). Thus, the developmentally expressed V_H gene segments mapped in this study are dispersed over a large fraction of the V_H locus. Moreover, since these V_H gene segments are interspersed with V_H elements that are not known to be developmentally expressed, we interpret our results to indicate that chromosomal location alone cannot explain preferential rearrangement and expression during fetal development.

Our data do not address mechanisms other than chromosomal location that may determine preferential fetal expression of specific V_H gene segments. Preference could be determined either by preferential targeting of the recombinase or by clonally selective mechanisms operating at the pre-B- or B-cell stage (targeted and selective mechanisms are not necessarily mutually exclusive). Because of their higher levels of immunoglobulin mRNA, small numbers of mature B cells in the fetal liver preparations used in the original analyses (4, 10, 11) would have contributed disproportionately to the V_H distribution in the cDNA libraries obtained, a result consistent with a selective mechanism of preferential expression. However, the small number of light chain recombinants in these cDNA libraries has been taken as evidence against the presence of mature B cells (4) and, by extrapolation, as evidence against a selective mechanism.

The V_H gene segments mapped in this study were each detected specifically by at least two independent oligonucleotide probes (Table 1). Gene segments identical to seven of the eight developmentally expressed V_H gene segments mapped in this study were isolated from a recombinant phage library. These results confirm the identity of the elements and confirm the sequence specificity of the oligonucleotide probes, validating their use in the deletional analysis. Two additional developmentally expressed V_H gene segments have been cloned from the germ lines of unrelated individuals. The 15p1 sequence and the 20p3 sequence have been found without substitutions in the population (14, 29, 30). These results indicate that developmentally expressed V_H gene segments are conserved and might encode specificities that are important early in development or might play an important role in the development of full immunocompetence.

Recently, Walter *et al.* (16) have determined a deletion map of the V_H locus in which the V_H gene segments are identified by restriction fragment size. Since, in the larger V_H gene families (V_H1 and V_H3), this approach does not allow identification of specific V_H gene segments it is difficult to compare their results with our map positions of the developmentally expressed V_H gene segments 20p1, 30p1, 38p1V, 51p1, 1.9III, hv3019b9, and 56p1. For the smaller V_H gene families (V_H2, V_H4, and V_H5) restriction fragment size can be diagnostic (15, 27). We assigned germ-line 58p2 to V_H4 band 12, which corresponds to V_H4-12 in the numbering by Walter *et al.* (15). Walter *et al.* (16) map V_H4-12 to a region between 780 and 880 kb 5′ of the V_H6 gene segment. This estimate corresponds well with our results, which place germ-line 58p2 at least 690 kb and maximally 890 kb 5′ of V_H6 (Fig. 3).

In the set of LCLs studied by Walter *et al.* (16), only one rearrangement extended beyond ≈800 kb. Similarly, we find the majority of rearrangements to occur within 700 kb 5′ of the V_H6 element and are able to map all developmentally expressed V_H gene segments within ≈1000 kb of J_H. Since the human V_H locus has been estimated to span 2500–3000 kb it seems somewhat surprising that rearrangements in Epstein–Barr virus-transformed B cells would be limited to V_H gene segments located in the 3′ proximal half of the locus. Although there are a number of plausible explanations for this observation, the simple interpretation is that the functional V_H germ line is confined to a region of ≈1000 kb spanning less than half of the total V_H locus. This region may contain fewer than 50 functional V_H elements. Since this possibility has important implications for the generation of the repertoire, it will be important to determine more directly the dimensions of the functional V_H germ line.

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