A Deletion Map of the Human Immunoglobulin Heavy Chain Variable Region

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Summary

Analysis of V_H gene segments deleted in the process of immunoglobulin heavy chain (IGH) variable region assembly in three series of monoclonal B cell lines has been used to determine the human V_H region organization. A deletion map of the relative positions of 21 different V_H gene segments has been determined. The characterization of B cell lines from three unrelated adults of two racial groups yielded the same relative V_H gene segment order, suggesting that the overall order of V_H genes in the normal population is constant. This V_H gene segment order was consistent with what we had previously generated from physical mapping techniques. D_H segments from the second D_H cluster, distinct from the major D_H locus 3' of the V_H region, were not observed to be used in 32 different rearrangements. Approximately 77% of the V_H-(D)J_H rearrangements involved V_H gene segments within 500 kb of the J_H region, indicating that human B cell lines preferentially rearrange J_H-proximal V_H gene segments. The switch, observed in mice, from the fetal use of J_H-proximal V_H gene segments to an adult V_H use dependent upon V_H family size may therefore not occur in humans. This detailed map of the V_H gene segments is a necessary prerequisite for understanding V_H usage in development and disease.

The human immunoglobulin heavy chain (IGH) gene complex is comprised of ~100 heavy chain variable (V_H) gene segments, at least 24 diversity (D_H) elements, six functional joining (J_H) segments, and a constant (C_H) region composed of nine genes and two pseudogenes. The IGH gene complex maps to the most distal band of chromosome 14, at 14q32.33 (1, 2). The order telomere-VH-JH-CH-centromere has been determined by analysis of Burkitt lymphoma cells having 8;14 translocations between the IGH locus and the oncogene *c-myc* (3).

The V_H gene segments, coding for the first 95–101 amino acids of the heavy chain peptide, have been subdivided into six families (V_H1 to V_H6) based upon DNA homology (4–9). The organization of portions of the V_H region has been determined from examination of cloned regions (10) and from long-range restriction mapping (4, 11). The human Ig V_H gene families are interspersed. This is in marked contrast to the murine V_H organization, which has some interspersion of the members of different V_H families, but appears to be characterized predominantly by the clustering of V_H families (reviewed in references 12 and 13).

The major D_H region, between V_H6 (the most 3' V_H gene segment) and the J_H region, is composed of four 9-kb intervals (14, 15). Each 9-kb repeating unit contains six different D_H gene families (16). A second D_H cluster is located within the V_H region (17, 18), but its functional significance, if any, is unknown. V_H, D_H, J_H, and C_H gene segments are juxtaposed in the course of B cell development. IGH variable region assembly is an ordered process that begins with D_H-to-J_H rearrangement, usually at both IGH alleles (19). The D_H-to-J_H complex then may recombine with a V_H gene segment. If the first IGH allele rearrangements does not produce an open reading frame, the second (D)J_H rearrangement can become a substrate for V_H-to-(D)J_H joining (reviewed in reference 20). Site-specific recombination of an upstream V_H gene segment into a V_H(D)J_H rearrangement, resulting in the replacement of the initially rearranged V_H gene segment, also can occur (21, 22). The frequency of this occurrence during normal B cell development is unknown. As the frequency of V_H-to-(D)J_H rearrangement is greater than the frequency of direct V_H to D_H rearrangements (23), control of Ig variable region assembly could be influenced by the accessibility of the rearrangement components to the recombination machinery.

In addition to D_H-to-J_H followed by V_H joining, direct D-D joining has also been suggested as potentially possible (14, 24). D-D joining was found infrequently by Meek et al. (60) in the analysis of IGH chain rearrangements from murine bone marrow. Ichihara et al. (25) found no evidence of direct D_H-to-D_H joining. However, the same group discovered a novel type of D_H element, diversity segments with irregular spacer lengths (DIR),[1] in sequencing 15 kb of the

[1] *Abbreviations used in this paper:* AAT, α_1-antitrypsin; DIR, diversity segments with irregular spacer lengths; PCFIA, particle concentration fluoroimmuno assays; PFGE, pulsed field gel electrophoresis.

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D_H region, which could be involved in D_H-to-DIR or DIR-to-D_H joining (16). While both mechanisms would result in the generation of increased diversity in the CDR III region of human Ig heavy chains, the frequencies of the occurrence of D_H-to-D_H and of DIR-to-D_H joining are unknown.

IGH variable region rearrangements result in the deletion of the intervening DNA that separated the rearranging V_H gene segment from the (D)J_H sequences (26). Deletion mapping takes advantage of this process to determine the relative positions of V_H gene segments. V_H gene segments 5′ of a selected V_H sequence remain unaltered by the rearrangement event, while those 3′ are deleted. This method has been used to elucidate the murine V_H organization (27–31).

We have used deletion mapping to determine the human V_H organization. We have examined 21 V_H gene segments of the V_H2, V_H3, V_H4, V_H5, and V_H6 families in three sets of monoclonal B cell lines from three different human donors. This analysis resulted in the generation of a V_H gene segment order consistent with the physical map we have derived from long-range restriction mapping experiments. Our results suggest that there is a single V_H gene segment order in humans, that interspersion of V_H gene segments is extensive, and that the human IGH recombination machinery generating adult B cell repertoires shows strong 3′ to 5′ bias.

Materials and Methods

B Cell Transformation. EBV-transformed human B cell lines were generated from PBMC of three unrelated normal adults as described (32, 33). Fifth-week supernatants were analyzed for the presence of secreted human IgG, A, M, D, E, κ, or λ by automated particle concentration fluoroimmuno assays (PCFIA), using a Baxter-Pandex ScreenMachine (Baxter, Pandex Div., Mundelein, IL) (34). Data were captured and processed using MacPlate PCFIA software developed in our institution. The frequencies of transformable cells committed to a given Ig isotype were determined by Poisson analysis by both minimum χ^2 and maximum likelihood procedures as described (32, 35). Secretory cultures, calculated by Poisson analysis to be monoclonal (initial cell dose containing $\leq$0.3 transformable cells) and producing a single Ig heavy and light chain, were sublined into 46 fresh microwells followed by recloning at limiting dilution (36). Clones were maintained in 100-cm^2 tissue culture flasks with complete medium. Phenotypic analysis by flow cytometry in a profile analyzer (Coulter Electronics, Hialeah, FL), followed standard procedures with commercial mAb reagents (Coulter Electronics) (32, 36).

Electrophoretic Analysis. High molecular weight DNA from peripheral blood samples of the B cell line donors was prepared in agarose plugs (37). DNA was prepared similarly from B cell lines, but cells were rinsed only once in PBS before embedding in agarose. Pulsed field gel electrophoresis (PFGE) analysis of DNA from HSC no. 1321 has been described (11; called there L1). HSC no. 1321 is of Caucasian descent; HSC no. 1001 and HSC no. 1322 are of Oriental descent. DNA was digested with 5–10 U of restriction endonuclease/μg of DNA in the manufacturer's recommended buffer (Boehringer Mannheim Biochemicals, Indianapolis, IN). Digested DNA samples (3 μg/lane) were fractionated through 0.7% agarose gels in a large submarine apparatus (Bethesda Research Laboratories, Bethesda, MD) at 60 V for 20–24 h. DNA was then transferred to Hybond N or N^+ membrane according to the manufacturer's recommendations (Amersham Canada, Oakville, ON). Blots were hybridized with ^{32}P-labeled DNA probes as described (37). After hybridization, blots were briefly rinsed in 2× SSC at room temperature and washed for 1 h in 0.1% SDS, 0.1× SSC at 52°C for the V_H2, V_H3f, V_H5, and AAT probes, or at 65°C for the J_H, D_H, V_H1, V_H4, and V_H6 probes. Filters were exposed to Kodak XAR-5 film at −70°C using intensifying screens (Lightning Plus; DuPont Co., Wilmington, DE).

DNA Probes. The hybridization probes used in this study were agarose gel–purified DNA fragments as follows: Sμ, the 2.2-kb SacI fragment from the switch region of Cμ, derived from λh18, originally isolated by P. Early and provided by R. Wall and L. Hood; J_H, a 6-kb BamHI/HindIII fragment derived from pHuJ(H) spanning the J_H region, provided by P. Leder (38); D_H, the 9.5-kb ClaI fragment of cosmid C17p3 (15); V_H2, the 1.2-kb BamHI/EcoRI insert of VH2BE1.2 (39), derived from VCE-1 (40) from T. Honjo; V_H3f, the 2.2-kb EcoRI fragment of VHE2.2 (39), which flanks the V_H3 family gene VH26 (41); V_H4, a 245-bp ApaI/EagI fragment derived from 58P2X, from H.W. Schroeder and R. Perlmutter (8); V_H5, a 221-bp PstI fragment derived from 2-V, from F. W. Alt (4); V_H6, a 9.3-kb BamHI fragment derived from cosmid C17p3 (15), containing the V_H6 gene segment; Cla20, a ClaI fragment (only 20 kb of which was cloned into C17p3) containing the V_H6 gene segment and flanking regions, derived from cosmid C17p3 (15). AAT is a 1.6-kb PstI fragment, subcloned from pATMB6.5 (42) containing exon II of the α_1-antitrypsin gene.

Densitometric Analysis. A computing laser densitometer (300A; Wise Molecular Dynamics) was used to compute fragment hybridization intensities in the B cell lines and controls, according to the manufacturer's suggested protocols. The hybridization intensities of 21 V_H gene segments in the DNA from each of the three sets of monoclonal B cell lines were compared with those in the donor's leukocyte DNA. Filters were rehybridized with a probe for the α_1-antitrypsin gene (AAT) to control for different amounts of DNA between lanes. Dosage of nonpolymorphic V_H gene segments deleted only on one rearranged chromosome was determined by both visual and densitometric analysis, the latter by averaging a minimum of three densitometric scans of each autoradiogram. Visual comparison and densitometric measurements differed in V_H dosage estimates in ~10% of the measurements. The V_H gene segment dosages in these situations were reassessed visually by an experienced independent person and dosage was then determined by the agreement of two of the three dosages estimates (two visual, one densitometric).

Restriction Map of the Human V_H Region. The physical map positions of the 21 V_H gene segments analyzed here were determined from the restriction map of a 1,500-kb region of the human IGH V_H region reported in reference 11, and included in Fig. 6. V_H gene segment designations were those of Walter et al. (11), based upon the relative positions (largest to smallest) of restriction fragments hybridizing to V_H family probes, in human DNA digested with EcoRI (for V_H3f-1) or BglII (for V_H2, V_H4, and V_H5 gene segments).

Results

Cell Lines. The culture efficiencies (sum of all transformable cells) in the three limiting dilution experiments (6.3–14.8% of B cells transformed) were within the range usually observed in our laboratory (33, 34, 43). Reflective of the Ig isotype commitment in circulating human B cell pools, ~85–90% of all transformants expressed IgM, ~5%

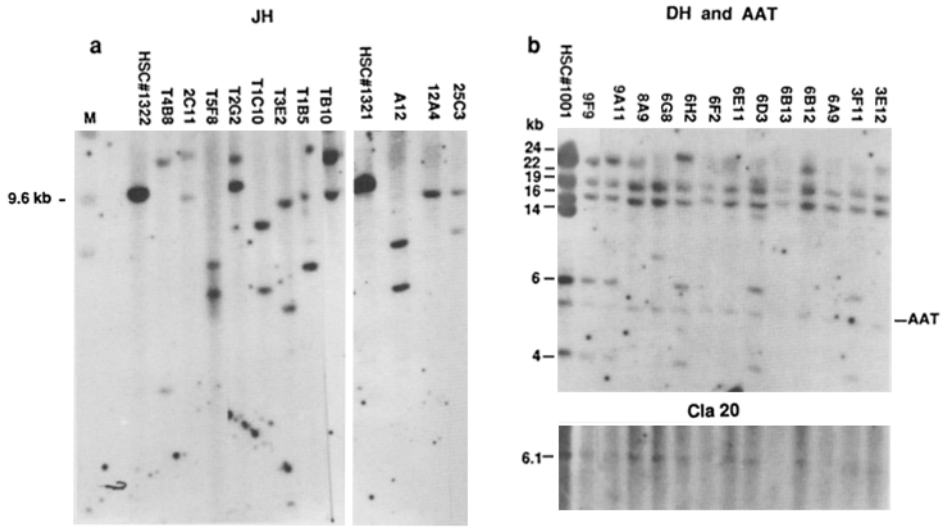

Figure 1. J_H rearrangements and D_H segments in selected B cell clones. (*a*) DNA from B cell lines and from the leukocytes of donors HSC no. 1322 and HSC no. 1321 was digested with HindIII, and hybridized with the J_H probe. The position of the J_H-hybridizing fragment detected in nonrearranged DNA is indicated. Sources of DNA separated in each lane are identified at the top. M, the positions of HindIII-digested λ DNA size markers. (*b*) EcoRI-digested DNA from B cell lines and from leukocytes of HSC no. 1001 was hybridized with the D_H probe (*top*), or the Cla20 probe (*bottom*). The hybridization of the Cla20 probe to the 6.1-kb EcoRI fragment is shown; the other fragments detected with Cla20 (as discussed in the text) either were also detected with the D_H probe or hybridized too faintly to allow dosage determination. The sizes of the D_H region fragments in nonrearranged DNA are indicated to the left. AAT, position of the AAT hybridizing fragment used as a control.

each expressed IgG or IgA, and ≤10% of IgM producers cosecreted IgD with 1–4% of these IgD producers coexpressing IgE. Of 42 lines derived from the three normal donors, 26 showed stable growth 6 mo after initiation, nine randomly selected clones had normal karyotypes, all had doubling times of ~48 h, and secreted their Ig at rates of 1–3 × 10^6 molecules/cell/h to a concentration of 1–10 μg/10^6 cells. None of the lines expressed T cell surface markers and all had the $CD20^+/CD21^+$ B cell phenotypes (36). None of the clones expressed CD5 but all were $CD23^+$. Therefore, the cell lines examined were EBV-transformed mature B cell clones.

Analysis of J_H Rearrangements. 26 different B cell lines, derived from the three different donors, were examined to determine if they were stable, monoclonal B cell lines suitable for deletion analysis. The B cell lines were digested with the restriction enzyme HindIII, and hybridized with the J_H probe (Fig. 1 *a*). The J_H probe hybridizes to a 9.6-kb HindIII fragment when not rearranged. Absence of this fragment or detection of novel fragments in DNA hybridized with the J_H probe indicates rearrangement of the J_H locus. By Southern hybridization, 19 of the 26 B cell lines examined in this manner appeared to have both J_H alleles rearranged (summarized in Fig. 2), six had retained one nonrearranged J_H allele. Information concerning the J_H region of B cell line 21A12 was not obtained, although later V_H and D_H analyses were consistent with the hypothesis that 21A12 had undergone two J_H rearrangement events. No lines showed identical patterns of rearranged IGH loci, demonstrating the independence of these B cell lines. Each B cell line contained only two J_H hybridizing fragments, consistent with the clonal nature of these cells. The B cell lines analyzed here were therefore concluded to be independent clonal B cell populations with stably rearranged IGH loci.

Analysis of D_H-to-D_H Rearrangements. To analyze the D_H rearrangements that the B cell lines had undergone in the process of differentiation, the 26 B cell lines were hybridized with a D_H probe, and with the Cla20 probe that extends 5′ of the D_H region. The B cell clones derived from HSC no. 1001 were hybridized with the D_H + AAT probes, and then with the Cla 20 probe (Fig. 1 *b*). The locations of the D_H, Cla20, V_H6, and J_H probes are shown in Fig. 2. The D_H and Cla 20 probes, together with the V_H6 probe and the J_H probe, survey the ~75-kb interval extending from the V_H6 gene segment to the J_H sequences (Fig. 2). The D_H probe detects a region of ~30 kb, hybridizing to fragments corresponding to the four 9-kb repeat elements that constitute the D_H major cluster (16). Additional fragments, belonging to the D_H minor cluster (discussed below), were also detected. The Cla20 probe detects the 24-kb EcoRI fragment of the D_H major cluster, and detects the 6.1- and 7-kb EcoRI fragments that are 5′ of the D_H major cluster (and also weakly hybridizes to the D_H minor region). However, only the 6.1-kb fragment hybridized with the Cla20 probe in a manner that allowed consistent determination of dosage. The D_H probe detects a common EcoRI polymorphism in the D_H region with alleles of 22 and 14 kb. The location of the polymorphic EcoRI site is indicated with an asterisk in Fig. 2. These 22- and 14-kb alleles have frequencies of 0.54 and 0.46, respectively, in the normal Canadian Caucasian population (Walter, M.A., and D.W. Cox, unpublished results). HSC no. 1322 is homozygous (22/22) for this D_H polymorphism, HSC no. 1001 is a heterozygote (22/14), and HSC no. 1321 is homozygous (14/14).

The results of the deletion analysis of the V_H6-J_H region of the 26 B cell lines are summarized in Fig. 2. 22 of the 26 B cell lines were found to have deletions in the D_H

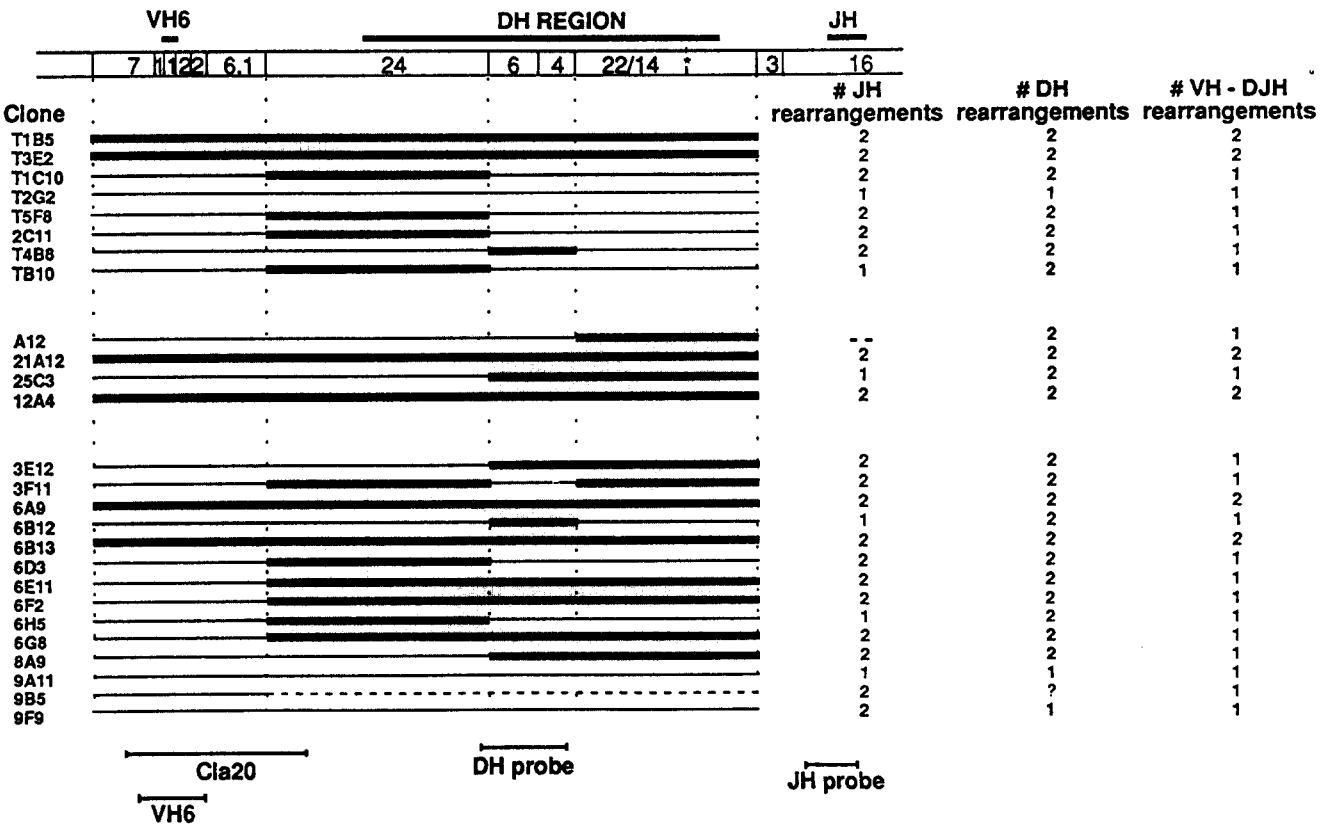

Figure 2. Summary of the D_H region deletions present in the B cell lines. The EcoRI restriction map of the V_H6-J_H region is indicated at the top, thin vertical lines indicate positions of EcoRI restriction sites. Sizes of EcoRI restriction fragments (kb) are indicated. (*) The position of a polymorphic EcoRI site in the D_H region. The positions of the V_H6 gene segment, D_H region, and J_H sequences within this DNA interval are indicated above the EcoRI restriction map, probes are mapped at the bottom. Thick lines indicate bi-allelic deletion of EcoRI fragments in a B cell clone. D_H region deletions on only a single chromosome in a B cell line were not determined. The horizontal dashed line indicates a region within the B cell line 9B5 that was not surveyed. The number of rearranged J_H alleles in a particular B cell line was obtained from Fig. 1. The number of V_H-(D)J_H rearrangements within a B cell line (*right panel*) was determined from the deletion mapping of fragments 5′ of the D_H region; in particular, the V_H6 gene segment and the 6.1-kb EcoRI fragment detected with the Cla 20 probe.

regions of both chromosomes when hybridized with the D_H and Cla20 probes (Fig. 2). Differences between the number of J_H rearrangements and D_H rearrangements were found in five B cell clones: TB10, 25C3, 6B12, 6H5, and 9F9. 9F9 appeared to rearrange only a single D_H allele, but two J_H alleles. 9F9 probably represents the rearrangement of two nonoverlapping portions of the D_H region, or alternatively, could have contained a rearrangement to the DHQ52 segment. DHQ52 is unusual as it lies within the J_H region (38). Nine B cell lines with portions of the D_H regions deleted on both chromosomes were found to retain DNA fragments that are 3′ of related D_H region sequences (T1C10, T5F8, 2C11, T4B8, TB10, 3F11, 6B12, 6D3, and 6H5). This suggests that in the 26 B cell lines, at least one half had IGH alleles that underwent D_H rearrangements distinct from D_H-to-J_H joining, likely the result of D-D rearrangement events. Since four of the B cell lines that appeared to retain one J_H allele in germline configuration (TB10, 25C3, 6B12, and 6H5), had rearrangements involving both D_H alleles (Fig. 2), D-D rearrangement events appear to occur rather more commonly than expected, and before D_H-to-J_H joining in Ig variable region assembly.

While most D_H sequence–containing fragments are rearranged or deleted in the B cell lines, two EcoRI fragments, ~19 and ~16 kb in size, are not (Fig. 1 *b*). These fragments persist in B cell lines in which several V_H gene segments have been deleted on both chromosomes (e.g., B cell line T1B5, see below). Examination of the restriction map of the 75-kb region between V_H6 (the most J_H-proximal V_H gene segment) (15) and the J_H region indicates that EcoRI restriction fragments of these sizes containing D_H homologous sequences do not lie in this interval (Fig. 2). These D_H fragments, therefore, appear to belong to the minor D_H cluster reported to map within the human V_H region (11, 17, 18) and were not involved in any of the Ig rearrangements analyzed here.

Analysis of 32 Different V_H-to-(D)J_H Rearrangement Events. Further analysis of the V_H6–J_H regions of the B cell lines revealed that, of the B cell lines with rearrangements of both D_H alleles (22/26), only six had V_H-to-(D)J_H rearrangements at both IgH alleles (T1B5, T3E2, 21A12, 12A4, 6A9, and 6B13), shown in Fig. 2. Most B cell lines had V_H-to-(D)J_H rearrangements of only one IGH allele, with the remaining V_H allele in the germline configuration (for example, B cell

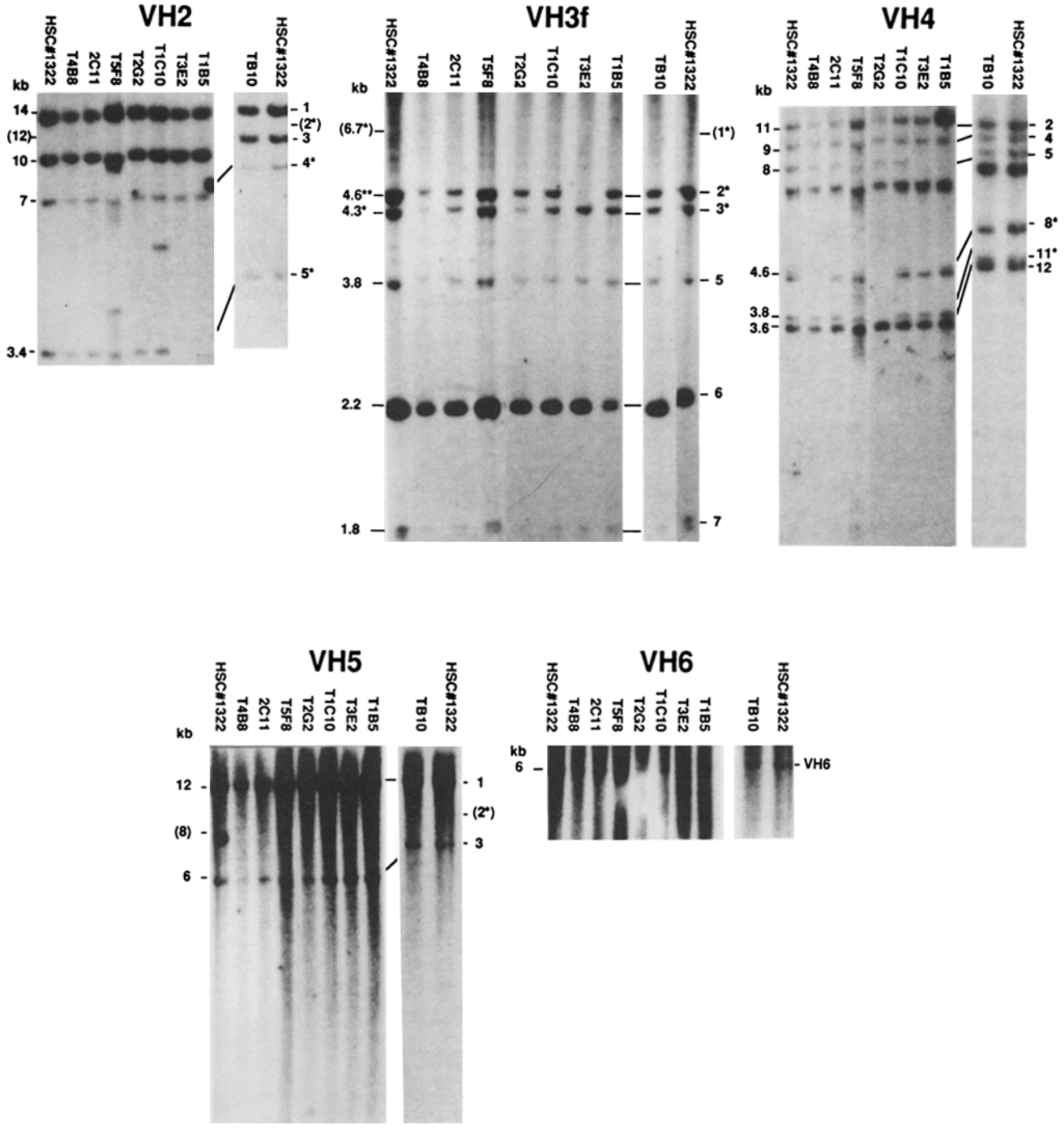

Figure 3. Analysis of V_H gene segments of the B cell lines derived from HSC no. 1322. All Southern blots were of BglII-digested DNA, except for VH3f (EcoRI). Sources of DNA separated in each lane are identified at the top. Blots were hybridized with the indicated V_H family probe. The V_H gene segments analyzed in the B cell lines are identified to the right. (*) The polymorphic V_H loci. Positions of V_H gene segments that were not present in the leukocyte DNA of HSC no. 1322 are indicated with parentheses. Sizes of V_H gene segments analyzed in this study are indicated to the left.

line T1C10). None of the B cell lines retained two nonrearranged IGH loci. We conclude that: (*a*) the 26 B cell lines analyzed here represent 32 different V_H-to-(D)J_H rearrangement events; and (*b*) that single allele V_H-to-(D)J_H joining seems to be common in human B cells.

Genetic Differences in V_H Gene Segment Repertoires. Knowledge of the polymorphisms present in the V_H region is crucial to the interpretation of deletion mapping results. The degree of genetic polymorphism in the human V_H region is high. Restriction fragment length polymorphisms (RFLPs) of the V_H2 (39, 44), V_H3 (39, 45–49), V_H4 (50; Walter and Cox, unpublished data), and V_H5 (51) families have been de-

Table 1. *Summary of the V_H Gene Segment Content of 26 B Cell Lines*

Clone	V_H2-1	V_H2-2	V_H2-3	V_H2-4	V_H2-5	V_H3f-1	V_H3f-2	V_H3f-3	V_H3f-5	V_H3f-6	V_H3f-7	V_H4-2	V_H4-4	V_H4-5	V_H4-8	V_H4-11	V_H4-12	V_H5-1	V_H5-2	V_H5-3	V_H6	Rearranged to:
T1B5	2	*	2	1	0	*	1	2	2	‡	ND	ND	2	0	**1**	**1**	2	2	*	2	0	ND
T3E2	1	*	2	1	0	*	0	2	2	‡	ND	2	2	0	**0**	**1**	2	2	*	2	0	VH3
T1C10	2	*	2	1	1	*	1	2	2	‡	ND	2	2	1	**1**	**1**	2	2	*	2	1	VH2
T2G2	2	*	2	1	1	*	1	1	2	‡	ND	1	2	1	**0**	**0**	2	2	*	1	1	VH5
T5F8	2	*	2	1	1	*	1	2	2	‡	ND	2	2	1	**1**	**1**	2	2	*	2	1	VH2
2C11	2	*	2	1	1	*	1	2	2	‡	ND	2	2	1	**0**	**1**	2	2	*	2	1	ND
T4B8	1	*	1	1	1	*	1	1	2	‡	ND	1	1	1	**0**	**1**	1	1	*	1	1	ND
2B10	2	*	2	1	1	*	1	1	2	‡	ND	2	2	1	**0**	**1**	2	2	*	2	1	ND
HSC no. 1322	2	*	2	2	2	*	2	2	2	‡	ND	2	2	2	**1**	**1**	2	2	*	2	2	
A12	2	**1**	2	2	*	**1**	2	2	2	2	2	2	2	1	2	**1**	2	2	1	2	1	VH2
21A12	2	**1**	2	1	*	**1**	0	1	2	0	2	ND	ND	ND	ND	**ND**	ND	2	ND	ND	0	ND
25C3	2	**1**	2	2	*	**1**	2	2	2	2	2	2	2	1	1	**1**	2	2	1	ND	1	ND
12A4	2	**1**	2	0	*	**1**	0	1	2	0	2	ND	ND	0	0	**1**	2	2	0	1	0	ND
HSC no. 1321	2	**1**	2	2	*	**1**	2	2	2	2	2	2	2	2	2	**1**	2	2	2	2	2	
3E12.	2	*	2	1	**1**	*	1	2	2	1	ND	ND	ND	ND	**ND**	**ND**	ND	2	**0**	2	1	ND
3F11	1	*	2	2	**1**	*	2	2	2	2	ND	ND	ND	ND	**ND**	**ND**	ND	2	**0**	2	1	ND
6A9	2	*	2	2	**0**	*	2	2	2	2	ND	ND	ND	ND	**ND**	**ND**	ND	2	**0**	2	0	ND
6B12	2	*	2	2	**1**	*	2	2	2	2	ND	ND	2	2	**1**	**1**	2	2	**1**	2	1	ND
6B13	1	*	2	2	**1**	*	ND	2	2	2	ND	ND	2	0	**1**	**1**	2	2	**0**	2	0	ND
6D3	2	*	2	2	**1**	*	2	2	2	2	ND	ND	2	2	**1**	**1**	2	2	**1**	2	1	ND
6E11.	2	*	2	1	**1**	*	1	1	2	1	ND	ND	2	1	**1**	**1**	2	2	**0**	2	1	ND
6F2	2	*	2	2	**0**	*	2	2	2	2	ND	ND	2	1	**1**	**1**	2	2	**1**	2	1	ND
6H5	2	*	2	1	**0**	*	1	1	2	1	ND	ND	2	1	**0**	**1**	2	2	**1**	1	1	ND
6G8	2	*	1	1	**0**	*	1	1	2	1	ND	ND	2	1	**0**	**1**	2	2	**1**	1	1	VH4
8A9	2	*	2	2	**1**	*	2	2	2	2	ND	ND	2	1	**1**	**1**	2	2	**0**	2	1	ND
9A11	2	*	2	1	**1**	*	1	2	2	1	ND	ND	2	1	**1**	**1**	2	2	**0**	2	1	ND
9B5	2	*	2	2	**0**	*	1	2	2	2	ND	ND	2	1	**1**	**1**	2	2	**1**	2	1	ND
9F9	2	*	2	2	**1**	*	2	2	2	2	ND	ND	2	1	**1**	**1**	2	2	**1**	2	1	ND
HSC no. 1001	2	*	2	2	**1**	*	2	2	2	2	ND	ND	2	2	**1**	**1**	2	2	**1**	2	2	
Percent DEL	8	0	4	33	50	0	38	15	0	25	0	11	2	55	40	5	2	2	55	10	62	

HSC no. 1322, HSC no. 1321, and HSC no. 1001 are the B cell line donors. 2, 1, and 0 indicate the number of copies of a particular V_H gene segment in a B cell clone. Numbers in bold indicate segregating V_H polymorphic loci.

Percent DEL is the percentage of times a V_H gene segment was observed to be deleted, versus the number of times that the particular V_H gene segment could have been deleted.

* The donor of the B cell line was homozygous for the deletion allele of an insertion/deletion V_H polymorphic locus.

‡ A rare polymorphism obscured the V_H3f-6 results of B cell lines derived from HSC no. 1322.

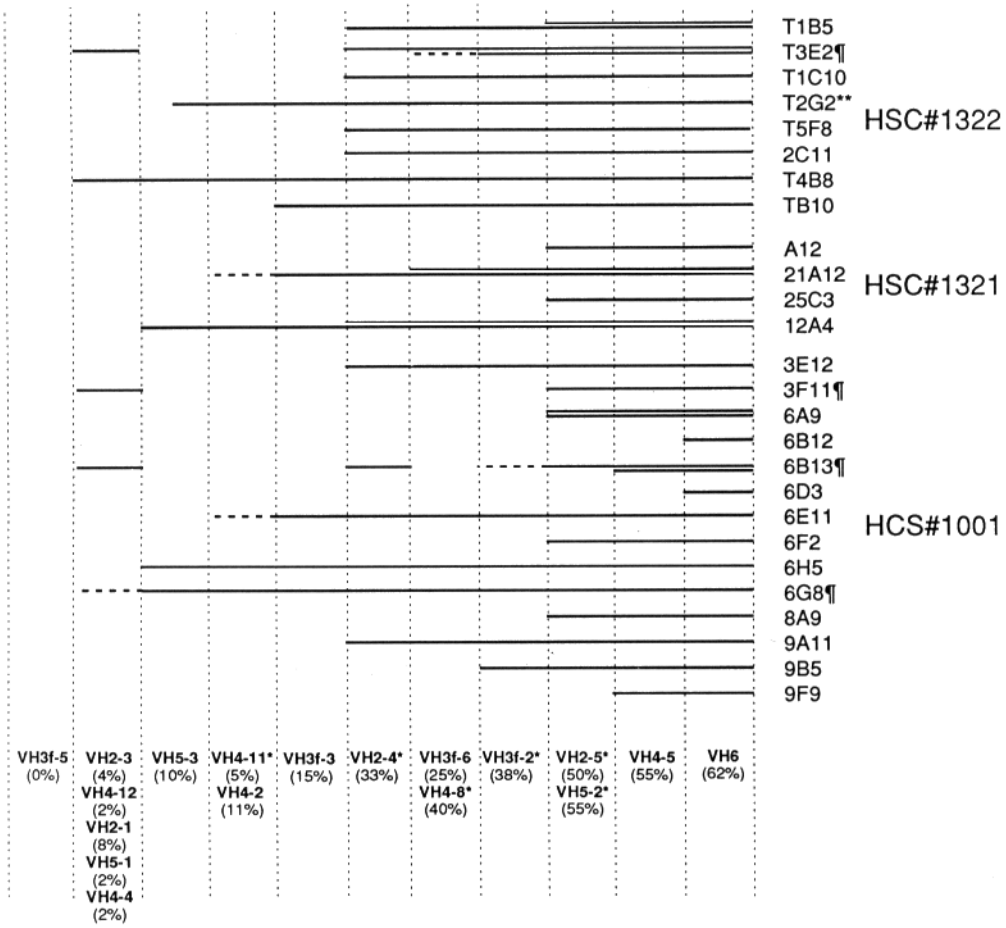

Figure 4. Summary of the V_H regions deleted in each of three sets of B cell lines. B cell lines are identified to the right, donors from which each set of cell lines was derived are indicated to the far right. Each thick black line represents one B cell line V_H-to-(D)J_H rearrangement event. B cell lines with two black lines have undergone two separate V_H-to-(D)J_H rearrangement events. The positions of the 21 analyzed V_H gene segments, as determined by deletion mapping, are indicated at the bottom of the figure. Numbers in parentheses, below each V_H gene segment, indicate the percentage of times that the V_H gene segment was deleted (indicated in Table 1). (*) The polymorphic V_H gene segments. The relative positions of V_H2-2, V_H3f-1, and V_H3f-5 are not indicated as they were not observed to be deleted in the four B cell lines in which they could be studied. Thin vertical dashed lines indicate intervals in which further ordering of V_H gene segments was not possible by deletion mapping. Horizontal dashed lines indicate that the B cell line deletion ends anywhere within the dashed area. (**) T2G2 appears to be rearranged to the V_H5-3 gene segment. (¶) Deletions in B cell lines T3E2, 3F11, 6B13, and 6G8 appear to be the result of nonlinear V_H-to-(D)J_H rearrangement events.

scribed. Most (13/15) of the characterized V_H region polymorphisms are insertion/deletion polymorphisms, indicating that the actual germline number of V_H gene segments differs between individuals. Examination of the DNA of the three donors reveals that no one donor has all human V_H gene segments (Table 1). Positional information of many of the polymorphic V_H gene segments could therefore be determined only through comparison, within each set of lines derived from each of the three donors.

Determination of V_H Gene Segment Dosage. DNA from three series of independent B cell clones, and from leukocytes of each donor, was analyzed for the presence or absence of V_H gene segments of five different human V_H families. The 21 V_H gene segments analyzed are shown in Fig. 3. The hybridization intensities of the 21 V_H gene segments in the DNA from each of the three sets of monoclonal B cells lines were compared by visual and densitometric examination with those in the donor's leukocyte DNA. Filters were rehybridized with another chromosome 14 probe, that for AAT, to control for different amounts of DNA between lanes. The results of the deletion analysis of 21 V_H gene segments detected with the V_H2, V_H3f, V_H4, V_H5, and V_H6 probes of all three series of B cell lines and their germline donors are summarized in Table 1. As 20 of the B cell lines had undergone only a single V_H-(D)J_H rearrangement event, in many cases a V_H gene segment was deleted on one IGH allele but not on the other. The regions deleted in the B cell lines are shown schematically in Fig. 4. B cell clones in which V_H probes detected novel fragments (discussed below) probably represent the V_H gene segments selected for V_H-to-(D)J_H joining (Table 1). The V_H gene segments that were rearranged in the other B cell clones could not be determined by the Southern hybridization analysis conducted here. In the B cell lines analyzed here with probes from the five V_H families, only the single member V_H6 family was not involved in any V_H-(D)J_H rearrangements.

Deletion Analysis of V_H2 Gene Segments. The V_H2 probe detects three polymorphic V_H2 loci in BglII-digested DNA (39, 44). V_H2-2 is an insertion/deletion polymorphism, with the presence or absence of a 12-kb fragment (absence indicated as 0 kb). V_H2-5 is also an insertion/deletion polymorphism, with alleles of 3.4 and 0 kb. V_H2-4 is a polymorphic locus with alleles of 7.3 and 7.0 kb. The V_H2 genotypes (V_H2-2, V_H2-4, V_H2-5) of the B cell line donors are: HSC no. 1322 (0/0, 7.0/7.0, 3.4/3.4); HSC no. 1321 (12/0, 7.0/7.0, 0/0); and HSC no. 1001 (0/0, 7.0/7.0, 3.4/0). V_H2-2, and

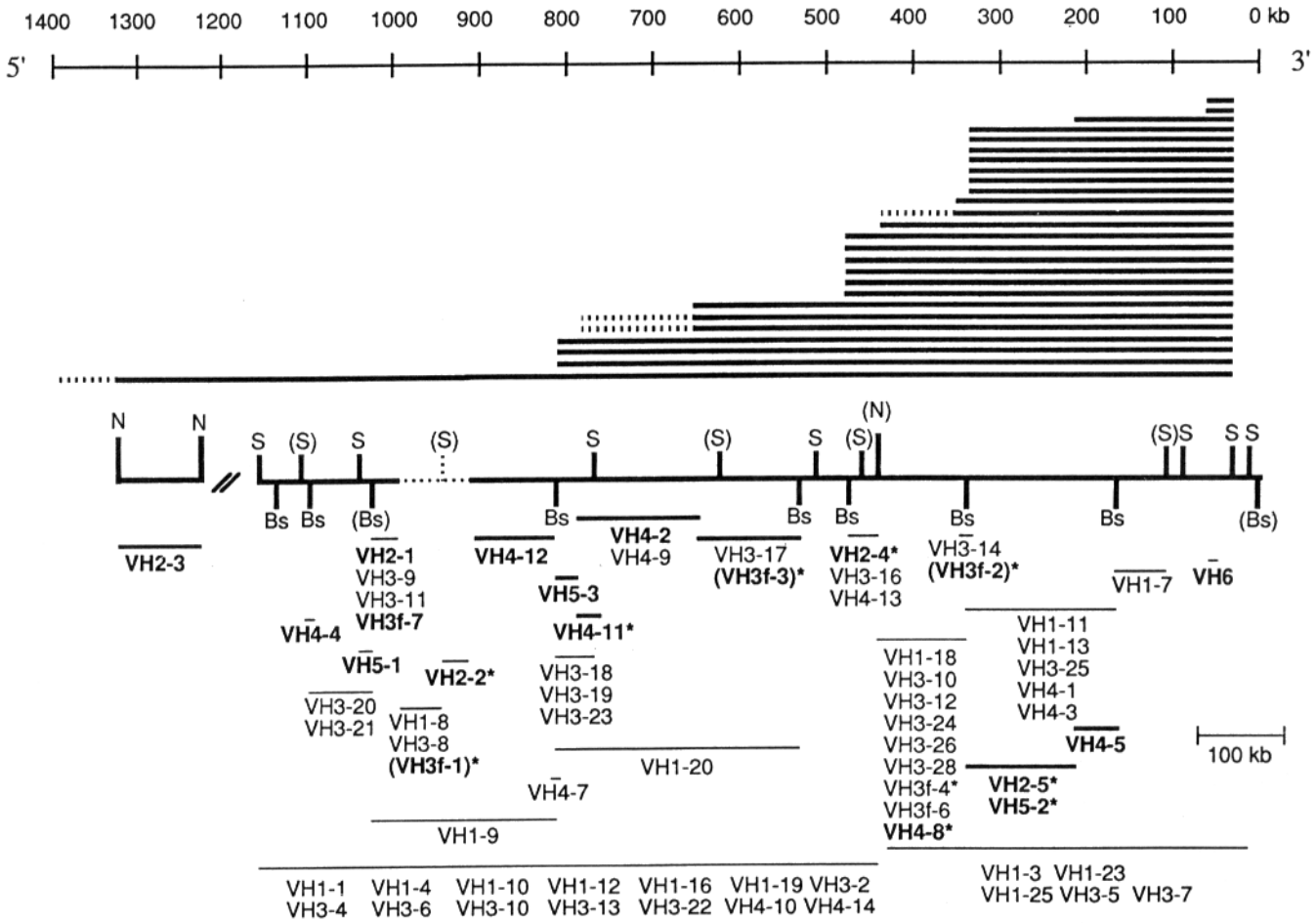

Figure 5. Comparison of deletion and physical mapping results in the V_H region. V_H gene segments in bold were characterized in both deletion and physical mapping experiments. Lines above the physical map indicate the alignment of the B cell line deletions from Fig. 4. Only the 26 linear V_H-(D)J_H rearrangements were included. Single nonlinear rearrangements in 3F11 and 6G8, and both rearrangements present in T3E2 and 6B13 were excluded, since it could not be determined which rearrangement in the latter two B cell lines were nonlinear. Physical mapping information is from Walter et al. (11). Bs, N, and S indicate positions of BssHII, NotI, and SfiI sites, respectively (parentheses indicate restriction sites that only partially digest in leukocyte DNA). The location of the common 80-kb insertion/deletion polymorphism, involving V_H2-2 and V_H3f-1, is indicated with a dotted line on the restriction map. The scale (in kb) is indicated at the top. Thicker horizontal lines indicate the locations of V_H gene segments further refined by deletion mapping results. The V_H2-3 positioning was determined from deletion mapping only. V_H3 gene segments also detected with the V_H3f probe are indicated in parentheses. (*) V_H polymorphic loci, usually of the insertion/deletion type.

V_H3f-1 (discussed below) are polymorphic V_H loci located within an 80-kb insertion/deletion polymorphism in the V_H region (11).

The deletions in the three sets of B cell lines are summarized in Fig. 4. The number of times that a V_H gene segment was deleted in the B cell lines, divided by the number of germline copies of the particular V_H gene segment, was used to determine relative V_H order. These values, expressed as a percentage, are shown in Table 1. In the V_H region rearrangements in the 26 B cell lines, V_H2-5 was deleted in 50% (15/30) of the opportunities in which this could have been detected. The V_H2-5 gene segment is only present on one chromosome of HSC no. 1001, and is not present at all in HSC no. 1321). V_H2-4 was deleted in 33% (17/52) of IGH alleles, V_H2-1 in 10% (5/52) of IGH alleles, and V_H2-3 was deleted in 4% (2/52) of IGH alleles. V_H2-2 was not observed to be deleted in any of the B cell lines but was present on only 4 of the 52 germline chromosomes examined. V_H2-2 was only present in HSC no. 1321, and in this individual, only heterozygously. Since these four B cell lines did not delete V_H2-3 or V_H2-1 (Table 1), the relative position of the V_H2-2 gene segment, 5′ of V_H2-4, could not be determined. Therefore, the V_H2 gene segment order, as determined by deletion analysis, is: 5′V_H2-3-V_H2-1-V_H2-4-V_H2-5-3′, with V_H2-2 located 5′ of the V_H2-4 gene segment. The deletion map obtained from the analysis of the 21 different V_H gene segments investigated in the 26 B cell lines is shown in Fig. 5.

The B cell line 6G8 appeared to delete V_H2-3, but not V_H2-1. This deletion order is not consistent with V_H gene segment order derived from the other B cell lines from HSC no. 1001 (the donor of 6G8) or from the other two donors. Therefore, either the dosage of these two V_H2 gene segments

was incorrectly determined in 6G8, or a nonlinear rearrangement event occurred in this B cell line (discussed below).

Novel fragments, not observed in the DNA of HCS no. 1322, were detected in the B cell lines TIC10 and T5F8 (Fig. 4). These novel V_H2 fragments were also detected with the J_H probe, suggesting that these B cell lines contained rearrangements to V_H2 gene segments. A novel fragment was also detected with the V_H2 family probe in the B cell line A12 (derived from HSC no. 1321), consistent with a rearrangement in this B cell line to a V_H2 gene segment (data not shown).

Deletion Analysis of V_H3 Gene Segments. Hybridization of B cell line DNA with V_H1 and V_H3 family probes yielded complex patterns of hybridizing fragments, precluding the identification of deleted V_H gene segments using these probes (data not shown). To simplify the hybridization pattern detected with the V_H3 family probe, we derived the V_H3f probe from the 5′ flanking region of the V_H3 gene segment V_H26 (11). V_H3f detects 7 of the ~28 V_H3 gene segments (11). The status of these seven V_H3 gene segments in the B cell lines was determined using the V_H3f probe. V_H3f-2 is a polymorphic locus with alleles of 6.0 and 4.6 kb in EcoRI-digested DNA (39). V_H3f-1, V_H3f-3, and V_H3f-4 are insertion/deletion polymorphisms, with alleles of 6.7 and 0 kb, 4.3- and 0 kb, and 3.5 and 0 kb, respectively in EcoRI-digested DNA. The V_H3 genotypes (V_H3f-1, V_H3f-2, V_H3f-3, V_H3f-4) of the B cell line donors are: HSC no. 1322 (0/0, 4.6/4.6, 4.3/4.3, 0/0); HSC no. 1321 (6.7/0, 6.0/6.0, 4.3/4.3, 0/0); and HSC no. 1001 (0/0, 6.0/6.0, 4.3/4.3, 0/0). In HSC no. 1322, the deletion status of the V_H3f-6 gene segment was not determined as it appeared that an additional V_H3 containing fragment comigrated with V_H3f-6 in this individual. V_H3f-5 was not deleted in any of the B cell lines, consistent with the previous suggestion that V_H3f-5 could lie outside of the V_H region (11). The intensities of V_H3f-2, V_H3f-3, and V_H3f-6 hybridizing fragments in the B cell lines derived from HSC no. 1321 were therefore determined by comparison with the hybridization intensity of the V_H3f-5 fragment. The insertion allele of the V_H3f-4 polymorphism (11) did not appear in the germline chromosomes of any of the three donors, precluding the ordering of this V_H3 gene segment.

The deletions in the three sets of B cell lines were analyzed to determine the V_H3f gene segment order, as was done for the V_H2 gene segments. This analysis indicates that the V_H3f gene segment order is: 5′-V_H3f-5-(V_H3f-1/V_H3f-7)-V_H3f-3-V_H3f-6-V_H3f-2- 3′. The relative order of the V_H3f-1 and V_H3f-7 gene segments could not be determined as no B cell line deleted one and not the other.

A novel V_H3 fragment was observed in the DNA of the HSC no. 1322 B cell line T3E2, detected with the V_H3 family probe. This novel fragment also hybridized to the J_H probe (data not shown), consistent with the rearrangement of the T3E2 B cell line to a V_H3 family gene segment. The complex pattern of hybridizing fragments detected by the V_H1 and V_H3 family probes precluded the detection of other rearrangements to members of these V_H families by Southern hybridization. The question as to whether any of the other B cell lines were rearranged to members of V_H1 or V_H3 families could be addressed by sequencing amplified V_H-(D)J_H regions.

Deletion Analysis of V_H4 Gene Segments. The V_H4 probe detects 14 V_H4 gene segments, many of which are polymorphic, and several of which lie on similarly sized BglII fragments (11, 50). Therefore, the deletion status of only the six clearly distinguishable V_H4 gene segments could be determined: V_H4-2, V_H4-4, V_H4-5, V_H4-8, V_H4-11, V_H4-12. Two of the V_H4 gene segments, V_H4-8 and V_H4-11, are insertion/deletion polymorphic loci, with alleles of 4.6 and 0 kb, and 3.8 and 0 kb, respectively, in BglII-digested DNA. The V_H4 genotypes (V_H4-8, V_H4-11) of the B cell line donors are: HSC no. 1322 (4.6/0, 3.8/0); HSC no. 1321 (4.6/4.6, 3.8/0); and HSC no. 1001 (4.6/0, 3.8/0). Analysis of the deletions present in the B cell lines yielded a relative V_H4 gene segment order of: 5′-(V_H4-4, V_H4-12)-(V_H4-2, V_H4-11)-V_H4-8-V_H4-5-3′. The relative order of V_H4-4 vs. V_H4-12, and of V_H4-2 vs. V_H4-11, could not be determined, as no B cell clone had deletions between the members of these pairs of V_H4 loci. The V_H4 probe detected a novel V_H4 fragment in DNA from the HSC no. 1001 B cell line 6G8 (data not shown), consistent with a V_H rearrangement in B cell line 6G8 to a V_H4 gene segment.

Deletion Analysis of V_H5 Gene Segments. The V_H5 probe hybridizes to the two or three members of the V_H5 family; the total number of V_H5 gene segments in humans is polymorphic (51). V_H5-2 is an insertion/deletion polymorphism with alleles of 8.0 and 0 kb in BglII-digested DNA. The V_H5 genotypes of the B cell line donors are: HSC no. 1322 (0/0); HSC no. 1321 (8.0/8.0); and HSC no. 1001 (8.0/0). Analysis of the B cell line deletions indicated a V_H5 gene segment order of: 5′-V_H5-1-V_H5-3-V_H5-2-3′. A novel fragment of 1.8 kb was detected by the V_H5 probe in BglII-digested DNA from the HSC no. 1322 B cell line T2G2 (Fig. 4). This same fragment hybridized to the J_H probe, indicating that T2G2 is rearranged to a V_H5 gene segment. The particular V_H5 gene segment used is probably V_H5-3, since HSC no. 1322 has only two V_H5 gene segments, and V_H5-1 is not deleted.

Deletion Analysis of the V_H6 Gene Segment. Hybridization of a V_H6 probe to B cell line DNA indicated that all B cell lines had deleted the V_H6 gene segment on at least one chromosome. In all B cell lines containing two V_H-to-(D)J_H rearrangement events, both V_H6 alleles were found to be deleted (T1B5, T3E2, 21A12, 12A4, 6A9, and 6B13; shown in Fig. 4). V_H6 was the V_H gene segment most often deleted: 63, 75, and 57% of the time in the B cell lines derived from HSC no. 1322, HSC no. 1321, and HSC no. 1001, respectively. None of the 32 different V_H-to-(D)J_H rearrangement events analyzed in the B cell lines were to the V_H6 gene segment. As V_H6 is the most 3′ V_H gene segment (4, 5, 15), the deletion model of V_H rearrangement predicts that V_H6 would be deleted whenever any other V_H gene segment is involved in a V_H-to-(D)J_H rearrangement. Analysis of the 32 different V_H-to-(D)J_H rearrangements in the 26 B cell lines

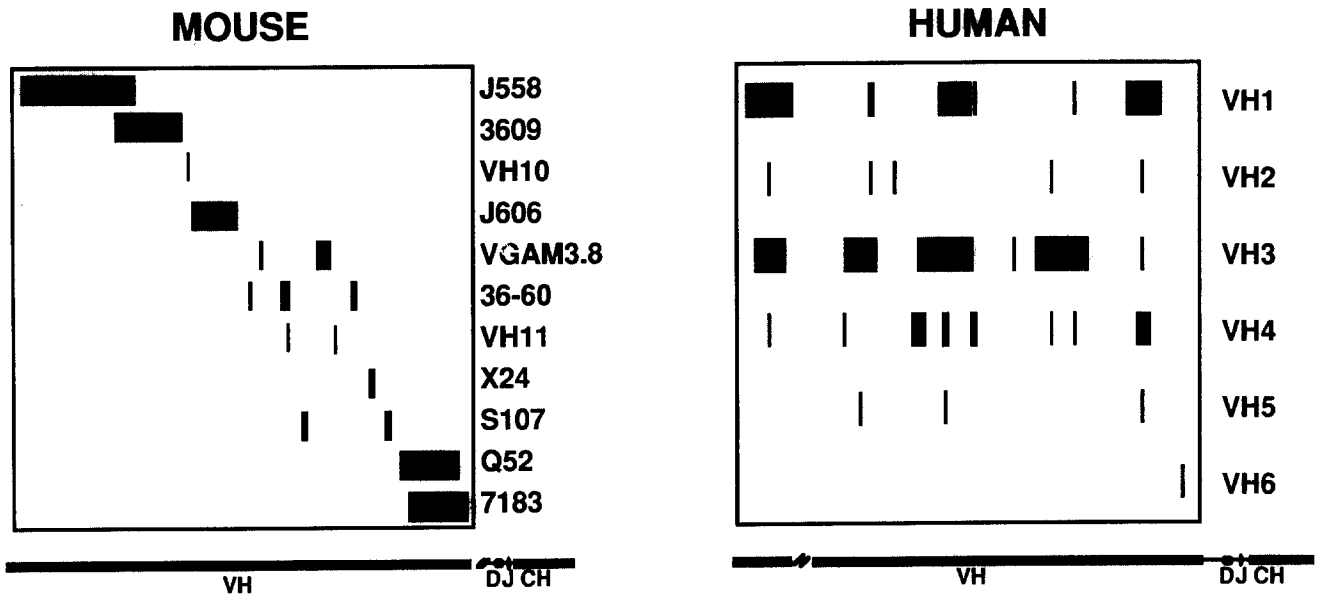

Figure 6. The organization of V_H gene families in mice and humans. The figure of the relative positions of the murine V_H families is adapted from Brodeur et al. Positions of the murine V_H10 and V_H11 families is from reference 31. The relative positions of the members of the human V_H families were obtained from Fig. 5. The number of V_H gene segments within each cluster is indicated by the lengths of the solid black bars. Broken lines within the IgH loci indicate regions that are not physically linked. The order of the V_H families does not indicate V_H family homology between mice and humans. Actual physical distances for the murine V_H region are unknown, the human V_H region is approximately to scale (the length of the long contiguous portion of the V_H region ~1,000 kb).

is consistent with this prediction. The V_H6 data are also consistent with the D_H region results, indicating that ~2/3 of the human B cell clones analyzed here were rearranged for a single IGH allele only, surprisingly different from the observations in rodent B cell lines, which usually undergo biallelic V_H region rearrangements (19, 23).

The Relative Order of V_H Gene Segments. The analysis of 21 V_H gene segments in the deletions generated through IGH rearrangements of the B cell lines indicates a relative order of V_H gene segments. In Fig. 4, the extent of the V_H region deletion present in each of the B cell lines are shown. The V_H gene segment order for each of the three sets of B cell lines was consistent with a single relative V_H gene segment order (Fig. 4). This deletion mapping V_H gene segment order is also consistent with the order derived by PFGE physical mapping techniques (11). This indicates that EBV-transformed B cells appear to maintain the germline V_H gene segment order, and that the V_H region rearrangement/deletion process appears to be mainly linear for V_H utilization. Exceptions to the linear rearrangement process are discussed below.

Comparison of the Results of Deletion and Physical Mapping Experiments in the V_H Region. In four regions, the V_H gene segment order from physical mapping studies (11) was further defined by deletion mapping results (Fig. 5). V_H4-5, V_H2-5, and V_H5-2 were mapped to the same interval by PFGE methods (11). B cell lines 6B13, 6D3, and 9F9 have deleted V_H4-5, but not V_H2-5 or V_H5-2. The order of those V_H gene segments therefore must be 5′-(V_H2-5, V_H5-2)-V_H4-5-3′. Similarly, B cell lines 6H5 and TB10 deleted V_H3f-3 but not V_H4-2, indicating that V_H3f-3 must be 3′ of V_H4-2. The B cell line T2G2 is deleted at the V_H4-11 locus, but V_H4-12 is retained. T2G2, as stated earlier, is rearranged to V_H5-3. The order of these V_H gene segments must be 5′-V_H4-12-V_H5-3-V_H4-11-3′. The V_H2-3 gene segment was mapped to a 100-kb NotI fragment, unlinked to the long range physical map of Walter et al. Deletion mapping results indicate that V_H2-3 was deleted in ~4% of rearrangement opportunities (Table 1). This is a frequency comparable with V_H gene segments V_H4-4, V_H4-12, V_H5-1, located at the 5′ end of the V_H region (11). The deletion mapping data are consistent with the positioning of the 100-kb NotI fragment (containing the V_H2-3 gene segment) near, and possibly adjacent to, the V_H4-4, V_H4-12, and V_H5-1 gene segments.

The relative V_H gene order, as determined by deletion mapping results, had been further defined in the PFGE-generated physical map of Walter et al., in four regions. V_H2-2 could not be positioned relative to V_H2-3, V_H2-1 and V_H3f-1, V_H3f-7; V_H4-4, V_H4-12; and V_H4-2, V_H4-11 could not be ordered by deletion analysis. The positions of these V_H gene segments had been more precisely determined within the V_H region though PFGE and 2D-DNA electrophoresis mapping to different large restriction fragments (11). The results of the physical and deletion mapping experiments were combined to generate the V_H gene segment physical organization shown in Fig. 5.

Examination of the V_H region organization presented in Fig. 5 reveals that the V_H gene segments of different V_H families are extensively interspersed. Many of the B cell lines (e.g., 9B5 and 21A12) deleted members of different V_H families while retaining other members of the same V_H families. No V_H gene family was observed to be exclusively deleted in any B cell line.

Nonlinear V_H-to-(D)J_H Rearrangements. A linear model of

IGH variable region assembly predicts that the V_H gene segments 3′ of a selected V_H gene segment will be deleted by the rearrangement process. While most (28/32) of the different V_H-to-(D)J_H rearrangement events analyzed in the B cell lines appeared to be consistent with this model, four different B cell lines (T3E2, 3E11, 6B13, and 6G8) appeared to retain V_H gene segments indicated by other B cell line rearrangements to be 3′ of V_H gene segments deleted in T3E2, 3E11, 6B13, and 6G8 (Fig. 4). For example, 3E12 is deleted for one copy of the V_H2-1 gene segment, but has two copies of all V_H gene segments between V_H3f-2 and V_H5-3. While errors in the determination of V_H gene segment dosage could have been made in these B cell lines, the nonlinear rearrangement events in T3E2, 3E11, 6B13, and 6G8 B cell lines could also be the result of other methods of V_H region rearrangement.

Utilization of 5′ End of the V_H Region. The 26 linear deletions of the V_H gene segments characterized in the B cell clones were aligned in Fig. 5. 62 of the 76 V_H gene segments that hybridized to the known V_H family probes were positioned within the physical map described in reference 11. Therefore, the ~1,000-kb portion of the V_H region analyzed in the B cell clones contains the great majority (at least 80%) of the V_H gene segment repertoire. V_H3f-3 represents an approximately "half-way mark" in the portion of the V_H region analyzed, being ~500 kb from the J_H region (11). 20 of the 26 rearrangements (77%) characterized in the B cell lines with only linear rearrangements involve V_H gene segments 3′ of, or in, the interval defined by V_H3f-3. Only six use V_H gene segments 5′ of V_H3f-3, and of these, only one extends beyond V_H2-3, the most 5′ V_H gene segment examined here. Thus adult-derived B cell lines preferentially use the V_H gene segments from the J_H-proximal V_H region.

Discussion

We have analyzed panels of monoclonal B cell lines from three different donors to elucidate the physical organization of the human Ig V_H region. The extent of the V_H region deletions that occurred during the rearrangement processes in the development of 26 independent B cell clones was characterized. This information was used to determine the relative positions of 21 V_H gene segments of the V_H2, V_H3, V_H4, V_H5, and V_H6 families, and allowed us to trace the search patterns of recombinase(s) involved in V_H-(D)J_H rearrangement processes in these B cells.

Honjo and Kataoka suggested a mechanism of IGH variable region assembly, later demonstrated by Cory and Adams, in which V_H gene segments 5′ of a selected V_H gene segment remain in the rearranged IGH locus while those 3′ are deleted. Deletion mapping takes advantage of this process. Several groups have used deletion mapping of V_H region rearrangements to generate a V_H family order for the murine V_H locus (27–31). Preliminary studies indicated that deletion analysis would also be possible in the human V_H region (17, 52). Our results indicate that deletion analysis is indeed a useful means of determining V_H gene segment organization in the large (>1,500 kb) human V_H locus.

Our results are largely consistent with the deletion model of IGH region rearrangement (26, 53). Consistent with previous observations in several human B cell lines (54), most of our B cell lines (19 of the 25 B cell clones for which J_H results were obtained) had bi-allelic J_H rearrangements. Of these lines, however, only six clones rearranged both V_H alleles. A recent report of V_H expression in EBV-transformed B cell lines (55) found that human B cell clones expressed heavy chain mRNA containing only one V_H gene segment. In murine B cell lines that have two V_H-(D)J_H rearrangements, both rearrangements frequently express detectable mRNA (56). These findings, together with the observation here that only 6/26 clones had two V_H rearrangements, leads us to conclude that most human B cells, unlike murine B cells, only undergo a single V_H-(D)J_H rearrangement.

While 28 of the 32 different V_H-to-(D)J_H rearrangements observed in the 26 B cell lines were consistent with a simple deletion method of rearrangement, 4 of the 32 V_H-to-(D)J_H rearrangements appeared to involve a different, secondary V_H rearrangement process. In these B cell lines (T3E2, 3F11, 6B13, and 6G8), V_H gene segments 3′ of deleted V_H regions were retained (Fig. 4). These rearrangements could be the result of an IGH V_H rearrangement method similar to, or the same as, the inversion/deletion processes observed in pre-B cells (57). An inversion rearrangement mechanism has also been shown to occur in the Vκ region (58), which allows the use of Vκ gene segments that are in the opposite transcriptional orientation to the Jκ and Cκ genes. The four nonlinear rearrangements that we observed in the B cell clones all involve the distal portion of the V_H region. The rearrangements in the B cell lines T3E2, 3F11, 6B13, and 6G8, therefore, could indicate that some of the V_H gene segments in the distal V_H region are in the opposite transcriptional orientation with respect to the C_H genes. However, at the level of Southern blot analysis, the majority (28/32) of the different V_H-to-(D)J_H rearrangement events appeared to be the result of single linear deletion events, suggesting that the transcriptional polarity of most V_H gene segments is the same as that of the C_H genes.

The V_H gene segment order as determined by deletion analysis was consistent with that determined by long-range physical mapping experiments (Fig. 5). This consistent V_H gene segment order also indicates that EBV-transformed B cell lines are suitable material for our studies. Different B cell clones use different V_H families, and rearrangements to gene segments of the V_H2, V_H3, V_H4, and V_H5 families were observed (Table 1). The B cell lines deleted different portions of the V_H region (Fig. 5). Selection during the course of EBV transformation for particular B cell sublineages, resulting in a bias in these B cell clones, is therefore not very probable. The relative V_H gene segment order was the same for all three B cell line donors, despite racial and extensive polymorphic V_H region differences between the three donors.

Since the first report of the discovery of D_H sequences, it has been postulated that D-D joining could be an additional means of generating antibody diversity (14, 24). D-D joining has been suggested as an explanation for long N regions (59). However, D-D joining would break the 12/23 rule of Ig rearrangement (20). Meek et al. (60) found direct evidence of

D-D joining, and detected both direct and inverted D-D recombination events. They suggest that a new recombination system would not be required for D-D joining, or alternatively, D-D joining could occur at low efficiency or use cryptic secondary heptomer/nonamer sequences. Ichihara et al. (25) found no evidence of D-D joining involving the human D_H segments, but later reported the discovery of a new type of D_H segment, which they termed DIR, whose spacer lengths were found to be irregular (16). They suggest that these DIR segments may be involved in D-D joining.

In the analysis of the D_H regions in the B cell clones studied here, 13/26 B cell clones had evidence of D-D joining (e.g., T1C10). Regions 3′ of portions of the D_H region that were deleted on both chromosomes of nine B cell lines were found to be present in at least one copy in the clones. Additionally, four of the clones (TB10, 25C3, 6B12, and 6H5) were observed to have two D_H region rearrangements, but only a single J_H rearrangement, consistent with the occurrence of D-D joining events before D-J joining. The frequency of the occurrence of D-D joining observed in these human B cell lines is much higher than that found in mouse B cells (estimated at 1/33,000; [60]). This could be the result of DIR segments, which have not been found in mice, or could indicate that the rearrangement processes in these two species are not identical.

Hybridization of a D_H region probe to DNA extracted from the 26 B cell lines revealed that two of the fragments were not deleted in the B cell lines (Fig. 1 *b*). As these fragments are homologous to the D_H region probe, but are not located within the major D_H region (between V_H6 and J_H), they define a second D_H locus. An additional D_H homologous region has been mapped to the IGH region of chromosome 14 (11, 17, 18), and may be the result of an unusual duplication event in the generation of the V_H locus in human evolution. However, a simple explanation of the events generating this second D_H locus is not possible, as neither the nearest V_H gene segment (V_H6), located 5′ of the major D_H cluster, nor the J_H region, located 3′ of the major D_H cluster, were duplicated in this putative event. It will be of interest to determine if, and when, any D_H sequences in this second D_H region are utilized in B cells, and if these cells define a distinct B cell sublineage.

Extensive genetic polymorphism involving the human V_H gene segments has been observed (39, 44–49, 51). Three sets of isogenic cell lines were studied to avoid problems due to uncharacterized genetic polymorphism. The number of members of a given V_H family varies between individuals in the normal population, since 13/15 characterized V_H polymorphisms involve insertion or deletion of V_H gene segments. Interestingly, despite this V_H gene number difference, the relative positions of the V_H gene segments in the three series of B cell lines are the same (Fig. 4). This implies that the order of V_H gene segments is the same for people with different sets of V_H polymorphic genes.

The order of V_H gene segments has been suggested to have functional significance. In mice, the J_H-proximal V_H gene segments were found to be preferentially used in fetal development (61–64). However, it has not been determined if the preferential use of the J_H-proximal V_H families is controlled by their position relative to the J_H region, or instead reflects fetal antigen selection of V_H families that might happen to be located in the 3′ V_H region. The concept of V_H use based upon V_H position alone no longer adequately fits the data. Murine strain differences in the use of V_H families suggest that other loci outside of the IGH complex have a role in control of V_H gene segment expression (65, 66). However, any preferential use of specific V_H gene segments in murine fetal development is thought to disappear over the course of murine development. The adult mouse appears to use V_H families in a manner more reflective of the size of V_H gene families, although there are inconsistencies with this theory as well (67).

In contrast to the detailed studies in the mouse, the pattern of human V_H gene segment expression is not as well characterized. Analyses of human fetal V_H expression have shown that members of all six V_H families are utilized (8, 68–70). However, attempts to correlate V_H gene segment expression with V_H position relative to the C_H region have not been possible in humans as these studies require a detailed map of the V_H region which, until very recently, has been unavailable. The extensive interspersion of the members of the various human V_H families have complicated methods to elucidate V_H organization. The recent generation of a physical map of the V_H gene segments by PFGE and two-dimensional DNA electrophoresis methods (11), and by the deletion mapping techniques in this report, should make possible comparison of human V_H gene expression with V_H location.

The organization of V_H gene segments in mice and humans is very different (Fig. 6). The analysis of the deletions present in the 26 B cell lines (Fig. 4) is consistent with cosmid cloning data (10), long-range restriction mapping (4, 11), and genetic analysis (39), all indicating that the human V_H families are extensively interspersed. This is in sharp contrast to the organization of the V_H region in the mouse, where the members of a given V_H gene family are generally clustered within the locus (12, 13), although examples of limited interspersion of some mouse V_H families has been shown (28, 29).

The different organization of the V_H loci between mouse and humans indicates that the evolution of the IGH gene complex occurred differently in these two species. It is difficult to imagine a mechanism that would result in the clustered V_H family organization seen in mouse strains, from an initial organization of interspersed V_H families, as found in humans. As well, no simple mechanism would generate the human interspersed V_H organization from an initially clustered V_H organization. Since human and mouse V_H regions must have undergone very different evolutionary processes to generate their current V_H repertoires, the control of V_H gene expression could therefore also be different between these two species.

The change in mice from preferential use of J_H-proximal V_H families in fetal development to V_H family use that is

more reflective of V_H family size in the adult, may not be necessary in humans, since the human V_H families are not clustered. Fig. 5 is a "footprint" of the recombinase that was active in the development of the B cell clones. 26 of the V_H region deletions characterized in the B cell lines have been aligned (the rearrangements present in the B cell lines with nonlinear rearrangements were not included). V_H3f-3 is located ~500-kb from the J_H region (11). The adult-derived B cell lines preferentially used V_H gene segments 3′ of, or in, the region containing V_H3f-3: V_H gene segments mapping within the J_H-proximal V_H region. This preference could represent selection for the expression of particular V_H gene segments during the development of the B cell precursors of our clones, before their function. As discussed above, selection for the expression of particular V_H gene segments in these B cell clones is unlikely. Instead, the B cell clones probably are representative of V_H gene segment usage in the circulating human B cell population. Members of all six human V_H families have members in the J_H-proximal V_H region (Fig. 6). Murine V_H organization, characterized by clustering of V_H families, may require a "normalization" mechanism to change from fetal to adult patterns of V_H use. In contrast, the recombination machinery in humans does not have to span large lengths of DNA in order to produce diverse antibody repertoires using V_H region gene segments from all V_H families. Preferential use of J_H-proximal V_H gene segments may therefore be maintained throughout human development.

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