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Complete amino acid sequence of the δ heavy chain of human immunoglobulin D

(antibody structure/diversity segment/joining region/lymphocyte receptor)

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ABSTRACT **We have determined the amino acid sequence of the variable (V) region of the δ heavy (H) chain of human IgD isolated from the plasma of myeloma patient WAH. This V region is unusual in its amino end group (arginine) and in its length (129 residues). The length is due to 10 insertions in the third complementarity-determining region (CDR3). A computer search showed that no reported CDR3–joining region (-J_H) sequences are identical and that they appear to be unrelated to the constant (C) region sequences of immunoglobulins. The V region sequence together with our previous results for the C region give the complete sequence of the human δ chain WAH, which has 512 amino acid residues and a $M_r \simeq 65{,}000$. The human δ chain has four domains (V, Cδ1, Cδ2, and Cδ3) and a long hinge region; by comparison, the mouse δ chain lacks a continuous segment of 135 residues, including half the hinge region and the entire Cδ2 domain. The human and mouse δ chains also differ in the number, kind, and location of GlcN and GalN glycans and probably in conformation and quaternary structure. These and other considerations suggest that there may be multiple forms of both secreted and membrane-bound IgD that differ in size, structure, and function.**

Although sequence data have been published for more than 50,000 amino acid residues of immunoglobulins of various classes and species, until recently (1–8) little was known about the amino acid sequence of human IgD. The reasons include: (*i*) the low concentration of serum IgD except in rare patients with multiple myeloma, (*ii*) the apparent lack of a characteristic antibody activity or effector function, (*iii*) the extreme sensitivity of IgD to "spontaneous" proteolytic degradation (1, 9), and (*iv*) the unusual structure of the hinge region, which presented difficult technical problems for its determination (5, 6). However, mounting evidence that this minor class of circulating immunoglobulins has a major function as an antigen receptor on the membrane of B lymphocytes (10, 11) led us to undertake determination of the complete primary structure of human IgD. We have reported the complete amino acid sequence of the Fc region (2) and of the Cδ1 and hinge regions (3), which together constitute the entire constant (C) region of the δ chain of the myeloma protein WAH. Here, we report the structure of the variable (V) region of the heavy (H) chain (the V_H region), which completes the amino acid sequence of the δ H chain; in work to be reported separately, we have determined the entire sequence of the λ light (L) chain, which completes the structure of a human IgD molecule.

Interest in the structure of IgD was stimulated by evidence that it functions as a major receptor on the surface of B lymphocytes, where it is coexpressed with IgM after antigen capture triggers cell differentiation (10, 11). Like IgM, IgD exists in two forms: sIgD, which is secreted into the serum, and mIgD, which is bound to the B-cell membrane. Cloning studies (12–16) have shown that the μ and δ structural genes may both be expressed in a single primary transcript that can be processed to yield either IgM or IgD having the same V_H region. Although the recent findings on the cellular role and biosynthesis of μ and δ chains are similar for mice and humans (9–16), and although human and mouse IgM are similar in structure (17, 18), the mouse δ chain is unusual in that it lacks the Cδ2 domain present in the human δ chain (1–7). The hinge structures of the δ chains of the two species also differ, and there is unexpectedly low homology in the Cδ1 domains (3). These results suggest that there may be several different forms of IgD in addition to the alternative forms of sIgD and mIgD that appear to differ only in their carboxyl-terminal tailpieces.

EXPERIMENTAL PROCEDURES

Methods described by Lin and Putnam (1) were used to purify WAH IgD and to prepare the tryptic Fab and Fc fragments of IgD [Fab(t) and Fc(t)], the papain Fab and Fc fragments of IgD [Fab(p) and Fc(p)], and the λ L and δ H chains. CNBr fragments were prepared (1–3) and were separated on Sephadex G-75 and purified by high-performance liquid chromatography (5, 6). Peptides were prepared by digestion with trypsin, chymotrypsin, or *Staphylococcus aureus* V8 protease (2, 3). The amino-terminal CNBr fragment of the δ chain was resistant to these enzymes and so was digested with pepsin and also cleaved by iodosobenzoic acid or dilute acid (0.03 M HCl). Peptide digests were separated by gel filtration on Sephadex G-50 and purified by high-performance liquid chromatography (4–6). The methods for amino acid analysis and sequence determination of the peptides with the Beckman model 890C sequencer have been described (1–6).

Whereas most human and animal heavy chains begin with a blocked end group (pyrrolidone carboxylic acid) or with glutamic acid (19–21), the WAH δ chain begins with arginine, which has not been reported previously as the amino end group of any heavy chain. Although Edman degradation of the intact

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Abbreviations: Fab(t) and Fc(t), tryptic Fab and Fc fragments of IgD; Fab(p) and Fc(p), papain Fab and Fc fragments of IgD. Abbreviations for classes, fragments, regions, and domains of immunoglobulins accord with official World Health Organization recommendations for human immunoglobulins published in ref. 34: CDR, complementarity-determining region; L, light; H, heavy; V, variable; V_H, variable region of H chain; J_H, joining region of H chain; D, diversity segment of H chain; C, constant region.

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δ chain was successful for only 10 steps, clear results were obtained for 42 steps with the amino-terminal CNBr fragment. The sequence of the segment from Gln-41 through Arg-68 was difficult to determine because the peptides in this region were obtained only in low yield by enzymatic digestion and chemical cleavage. All positions in the V region were established by at least two independent methods, and all required overlaps of peptides were obtained.

RESULTS AND DISCUSSION

Length and M_r of the δ Chain. The WAH δ chain has the longest V region sequence yet reported for human immunoglobulins (i.e., 129 amino acid residues compared to 126 in the Ou μ chain (17)—the longest previously recorded). Taken together, the sequences of the V region, the Cδ1 domain (2), the hinge region (2), and the Fc region (3) yield the complete amino acid sequence of the δ chain of IgD WAH, including the identification of all acids and amides and the sites and nature of the oligosaccharides (Fig. 1). The entire δ chain contains 512 amino acid residues; the M_r of the polypeptide portion is 56,213. The three GlcN glycans and the four (or five) GalN glycans contribute a total M_r of ≈9,000; thus, $M_r \simeq 65{,}000$ for the human δ chain, which accords with our earlier estimate of 66,000 ± 1,000 (1). Previous estimates have ranged from $M_r \simeq 60{,}000$ to $M_r \simeq 69{,}000$, which led to suggestions that the δ chain might contain four C region domains (9, 10). However, our sequence determination shows that there are only three C region domains (Cδ1, Cδ2, and Cδ3) and that it is the extended length of the δ hinge that makes the δ chain intermediate in size between the μ and ε chains on one hand and γ and α on the other.

Structural Model of Human IgD. Because we also have determined the amino acid sequence of the λ light chain of WAH IgD (unpublished data), we can construct a structural model for human IgD. In this model, the light chain is linked by a disulfide bond to Cys-C15 in the Cδ1 domain, and the two heavy chains are joined by a single disulfide bridge at Cys-C161 at the end of the hinge to form a tetrachain monomeric molecule. IgD is the only human immunoglobulin class in which the two heavy chains are joined by a single disulfide bridge. In gel filtration, WAH IgD behaves as a four-chain monomer; the Fc fragment is a dimer because the disulfide bond linking the heavy chains remains intact after limited cleavage with papain or trypsin (1).

Special Characteristics of the V Region. Although the V region of the WAH δ chain has a unique amino end group (arginine), a high concentration and clustering of aromatic amino acids, and exceptional length, it clearly belongs to the V_{HII} subgroup of heavy chains, for it has 37 of the 41 residues assigned by Kabat *et al.* (20) as "invariant" in subgroup II. The WAH δ chain is notable for the clustering of aromatic residues in the first complementarity-determining region (CDR1), where the sequence Tyr-Tyr-Trp-Gly-Trp occurs (see Tyr-34) and in CDR2, where there are two Tyr-Tyr sequences (see Tyr-54 and Tyr-60); in addition, there is one Tyr-Tyr sequence in CDR3 (see Tyr-105). There is a similar clustering of aromatic residues in CDR1 of the λ L chain, where the sequence Tyr-Val-Tyr-Trp-Tyr occurs. Thus, the CDR regions of this IgD protein epitomize the "ring-of-rings" identified by Edmundson *et al.* (22) as important determinants of combining specificity particularly for aromatic ligands.

Length of the CDR3 and J_H Regions. The greatest variability in amino acid sequence in H chains occurs in the third hypervariability region, earlier called HV3 or the hypervariable deletion region (19) and now often called CDR3 (20). Differences in length of the V region are primarily determined by the CDR3 region, which is also the major determinant of the size and shape of the antibody combining site and of idiotypic determinants (19, 22, 23). CDR3 and the joining segment (J_H) make up the carboxyl-terminal portion of the V_H region. The CDR3 region of a H chain is encoded by a separate DNA segment called the diversity (D) segment, and two recombination events (V_H-D and D-J_H joinings) are necessary to form a complete *V* gene (24, 25). The boundaries of the J_H and CDR3 segments also have been predicted by comparison of a series of amino acid sequences (26). There are many J_H amino acid sequences, but the prototype sequence is X-X-Trp-Gly-Gln-Gly-Thr-X-Val-Thr-Val-Ser-Ser in which X represents positions where substitutions most often occur. Kabat *et al.* (20) set the boundaries of the CDR3 region as V95–V102 in their numbering system and made provision for possible insertions to be numbered as 100A, 100B, etc. For human heavy chains, Andrews and Capra (27) showed that the length of CDR3 varied from 7 to 17 amino acid residues. By the same criteria, the length of CDR3 in the WAH δ chain is 19 residues; thus, the WAH δ chain is unique in the unmatched length of its CDR3 region.

Genetic Origin of the D Region Encoding CDR3. With one apparent exception, all known DNA exons for immunoglobulin chains code precisely for functional segments of the polypeptide chains (e.g., the exons for the V and J segments and for each C region domain in H and L chains and the exons for the tailpieces of the secreted and membrane forms of μ chains). The exception appears to be the DNA segment for the D (or CDR3) sequence. Potential germ-line sequences for the D segment are just beginning to be reported for mouse (24) and human (25) DNA. Their size is variable and is smaller than the usual CDR3 region, and their number in the germ-line is unknown. Recent evidence suggests that the human D segments are encoded in tandem multigene families and that they may recombine and be read in different frames (25).

Because of the uncertainty about the genetic origin of the D region encoding CDR3, we requested Winona C. Barker of the Atlas of Protein Sequence and Structure to use its programs (21, 28) to make a computer search of all reported amino acid sequence data for segments homologous to the 30-residue section of the WAH δ chain from positions 100 to 129 that represent CDR3 and J_H. We expected no identical sequence, and there was none. Of almost 210,000 segments of 30-residue length compared, the first 41 having nine or more identities included the CDR3–J_H area of the V region of immunoglobulin chains; most of the identities were in the J area, and the sequences all terminated at the end of the V region. To our surprise, the highest score (14 identities) was for two mouse H chain V regions (T601 and MOPC315) (Fig. 2). Human and mouse H chain V regions were about equally represented for scores of 13 and 12, and dog and rabbit H chains were among those scoring 11. With the exception of one human λ chain V region, all segments scoring 10 were H chain V regions of various species. No significant relationship was found between the CDR3–J_H sequence of the WAH δ chain and the C region sequence of any immunoglobulin. Hence, we conclude that neither CDR3 nor J_H have any evolutionary relationship to genes coding for the C region. Furthermore, the degree of homology in the CDR3–J_H region is unrelated to the species, class, or subgroup of the H chain. Also, when the computer search was made with the genetic code matrix (21, 28), the ordering in the scores of the H chains was very similar to that shown in Fig. 2.

Location, Structure, and Function of Oligosaccharides. Human IgD contains about 12% carbohydrate; this is all bound to the δ chain and consists of three large GlcN oligosaccharides linked to the Fc region and four or five GalN trisaccharides in close proximity in the hinge region. The GlcN is always N-linked to an asparagine in the obligatory tripeptide acceptor

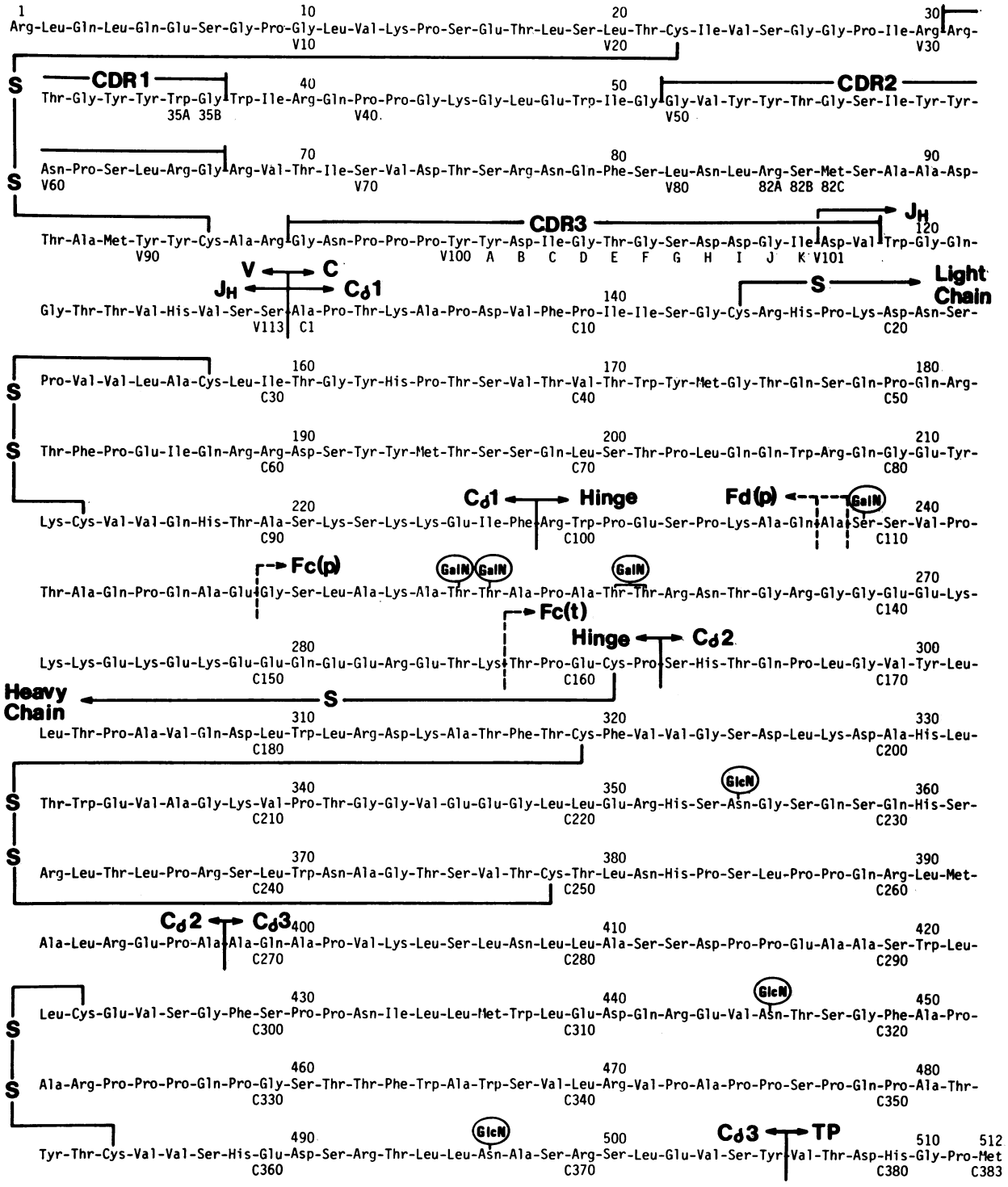

FIG. 1. Complete amino acid sequence of the δ chain of human IgD WAH. A double numbering system is used; the upper set represents the actual position beginning with the amino terminus. In the V region, the lower set accords with the computer-adjusted system of Kabat *et al.* (20), which takes account of insertions (numbered A, B, etc.) in the CDR. In the lower set, the C region residues are designated C and are numbered consecutively beginning with Ala-C1. The hinge region has four (or five) GalN oligosaccharides attached, and the Fc region has three GlcN oligosaccharides. Points of limited cleavage by papain (p) or trypsin (t) are shown by dash lines. The probable boundaries of each domain and of J_H, the hinge region, and the tailpiece (TP) are shown.

sequence Asn-X-Thr/Ser, where X may be any amino acid but rarely is proline or aspartic acid (29). All such sites in the WAH δ chain have GlcN attached; none is present in the WAH λ light chain. Jacques Baenziger of Washington University of St. Louis is determining the structure of the GlcN oligosaccharides on glycopeptides we isolated from the WAH δ chain. Probably one high-mannose and two complex GlcN glycans are present.

The purification and sequence analysis of the GalN glycopeptides was difficult because the GalN-rich portion of the δ hinge region was resistant to proteolytic cleavage because of the close proximity of the carbohydrates. The procedures for determination of the amino acid sequence and location of the carbohydrate are given by Takahashi *et al.* (6), who found that one GalN is linked to the first serine in the sequence Ala-Ser-Ser

	Heavy chain			Score	Residues		CDR3 ←	J$_H$ →
Hu	δ	II	WAH	30			G N P P P Y Y D I G T G S D D G I	D V W G Q G T T V H V S S
Mo	α	III	T601	14	89	118	E D T A L Y Y C A R L G Y Y G Y F	D V W G A G T T V T V S S
Mo	α	I	MOPC315	14	107	136	Z B T A T Y Y C A G D N D H L Y F	D Y W G Q G T T L T V S S
Hu	γ1	II	Newm	13	88	117	A D T A V Y Y C A R N L I A G C I	D V W G Q G S L V T V S S
Hu	γ2,μ	III	Til	13	86	115	D T A V Y Y C A K G K V S A Y Y F	B Y W G Z G T L V T V S S
Hu	μ	III	Ga	13	93	122	V Y Y C A R S G I A L G S V A G T	D Y W G Z G T L V T I S S
Mo			X24	13	88	117	E D T A L Y Y C A R L G Y Y G Y F	D Y W G Q G T T L T V S S
Mo	α	III	TEPC15*	13	94	123	A I Y Y C A R D Y Y G S S Y W Y F	D V W G A G T T V T V S S
Mo	μ	II	104E	13	88	117	S E D S A V Y Y C A R D Y D W Y F	D V W G A G T T V T V S S
Hu	γ1	II	Daw	12	90	119	G D T A T Y Y C A R S C G S Q Y F	D Y W G Q G I L V T V S S
Hu	γ1	II	He	12	92	121	D T A T Y Y C V H R H P R T L A F	D V W G Q G T K V A V S S
Mo			MPC11	12	92	121	A I Y H C A R G I Y Y N S S P Y F	D S W G Q G T T L T V S S
Mo			MOPC141#	12	115	144	A S V S I Y Y Y G R S D K Y F T L	D Y W G Q G T S V T V S S
Mo	α1	III	MOPC511	12	95	124	I Y Y C A R D G D Y G S S Y W Y F	D V W G A G T T V T V S S

FIG. 2. Homology of the CDR3 and J$_H$ segments of human (Hu) and mouse (Mo) immunoglobulin H chains as aligned by a computer comparison of the 30-residue sequence of the CDR3 and J$_H$ segments of the WAH δ chain with the entire library of amino acid sequence data reported for all proteins. The one-letter notation for amino acids is given in ref. 21. The score gives the number of identities with the CDR3–J$_H$ sequence of IgD WAH. Sources of the immunoglobulin sequences are from standard atlases (20, 21). Roman numerals specify the V region subgroup (19). The circled C denotes the second cysteine in the V region intrachain disulfide bridge. The overlapping boundaries of CDR3 and J$_H$ probably result from codon variation in selection of the second and third nucleotide bases in the DNA palindromes (24, 25) that are present at the 5′ and 3′ ends of encoding sequences.

and one to each of the threonines in the first Ala-Thr-Thr sequence. It is uncertain whether the second Ala-Thr-Thr sequence has GalN on both threonines or only one. In contrast, in IgA1, the only other human immunoglobulin that contains GalN, the five GalN-Gal disaccharides present in the α1 hinge are all linked to serine in the repeating sequence Pro-Ser (30, 31). In both the δ and α1 chains, the site of GalN attachment is probably determined both by the hydroxyamino acid and by the neighboring secondary structure.

Comparison of Human and Mouse IgD. Although the μ chains of human and mouse IgM are very similar in domain structure and in amino acid sequence, the δ chains of human and mouse IgD differ in several major characteristics. The most important of these is the apparent absence of the Cδ2 domain in mouse IgD (12, 13). In addition, the hinge region of the mouse δ chain is half the length of the hinge region in the human δ chain, and the two chains differ in the number, kind, and location of their oligosaccharides and in the size and sequence of their tailpieces. These major structural differences of the human and mouse δ chains are illustrated schematically in Fig. 3, which shows a deletion of a continuous segment of about 135 amino acids corresponding to the absence in mouse δ chain of the Cδ2 domain and of the COOH-terminal half of the human δ chain hinge (i.e., the high-charge segment) (12, 13, 32). The mouse δ chain also lacks the cysteine residue present at the COOH terminus of the human δ chain hinge, which crosslinks two H chains to form an IgD monomer. Hence, mouse IgD may exist as half-molecules; indeed, an HL structure has been proposed for one form of murine cell surface IgD (33). Such a half molecule lacking the Cδ2 domain and part of the hinge region would differ greatly from human IgD in molecular conformation and, thus, in the way a signal could be transmitted between the antibody combining site in the V region to the membrane binding site at the distal end.

Human and mouse IgD differ from other classes of immunoglobulins, such as IgG and IgM, in which the number and location of oligosaccharides are similar in the mouse and human homologues. Whereas human IgD has four (or five) GalN glycans in the first half of the hinge, the mouse δ hinge has one GlcN glycan (32). The lack of GalN in the mouse δ hinge may

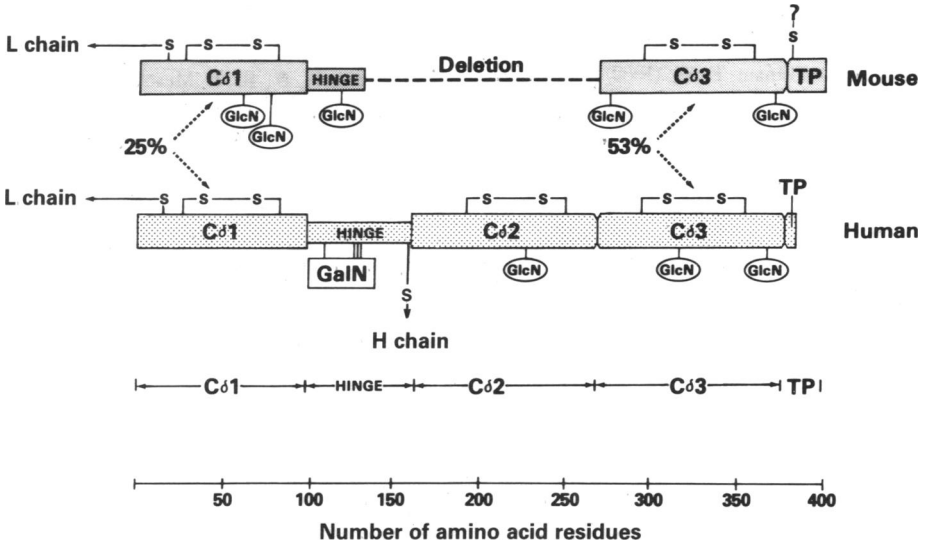

FIG. 3. Structural models of the δ chains of human and mouse IgD. The model for human δ chain is from this work. The model for mouse δ chain is based on the updated DNA sequence of Cheng *et al.* (13) and the partial protein sequence of Dildrop and Beyreuther (32). The Cδ1 domains of the two species have about 25% identity in amino acid sequence, whereas the Cδ3 domains have about 53% identity. TP, tailpiece.

be due to the absence of the characteristic GalN attachment sites of Ala-Thr-Thr and Ala-Ser-Ser in the human δ hinge (Fig. 1) and of Pro-Ser in the human α1 hinge (30, 31). Of the six Asn-X-Thr/Ser acceptor sites for GlcN predicted from the updated DNA sequence of mouse δ chain (13), five are glycosylated in the IgD protein (32). This compares with three GlcN glycans in the human δ chain, all of which are in the Fc region. Only one of the GlcN oligosaccharides in the human and mouse δ chains is at the same site (i.e., the one at the COOH terminus).

One of the most puzzling differences in the two δ chains is in the tailpiece. Whereas there are only two amino acid changes in the 20-residue tailpiece of the human and mouse μ chains of secreted IgM (14), there is no structural relationship between the tailpieces of the secreted forms of human and mouse IgD. The amino acid sequence of the tailpiece of the secreted mouse IgD protein is the same as that deduced from the DNA sequence as corrected by Cheng *et al.* (13). Although the entire Fc sequence is identical in the two human IgD proteins for which it has been reported—i.e., WAH (2) and NIG-65 (7)—the length of the tailpiece in these δ chains is only 6 amino acid residues compared to 20 in the mouse δ tailpiece. Multiple forms of the tailpieces of the human δ chain have been reported (16); some though not all of the differences are attributed to the degree of glycosylation, but neither amino acid nor DNA sequence data are available. Several distally coded exonic segments that are potential alternate carboxyl termini for the mouse δ chain have been identified, but their function is unclear (12, 13). Taken together, these results suggest that there may be multiple forms of both secreted and membrane IgD that differ in size, structure, and function.

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