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Immunology

Primary structure of the Fc region of human immunoglobulin D: Implications for evolutionary origin and biological function

(domain structure/homology comparison/evolutionary tree/gene duplication/lymphocyte receptor)

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ABSTRACT **We have determined the complete amino acid sequence of a tryptic Fc_δ fragment generated from an intact human IgD (WAH); it is 226 residues long and includes the second ($C_\delta 2$) and the third ($C_\delta 3$) constant domains of the δ chain. Comparison of the homology of the Fc sequence of the five human immunoglobulin classes suggests that either the δ-chain gene evolved from the α-chain gene soon after the divergence of a μ-α common ancestor or it evolved from an ancestral gene distinct from both the μ-α and the γ-ε common ancestors. Comparative study using a spatial model of the Fc region indicates that the structure of the $C_\delta 3$ domain differs extensively from that of the carboxy-terminal domains of other heavy chain classes; this, together with the unique hinge region structure, probably reflects the biological role of IgD as a receptor molecule on the B-lymphocyte surface.**

Immunoglobulin D exists in two different forms: the serum IgD and the membrane IgD (1). Serum IgD represents only a very minor fraction of the total serum Ig, and it confers no particular antibody activities or effector functions (2). On the other hand, the coexistence of membrane IgD with IgM on the surface of mature B cells has led to the general recognition of membrane IgD as a lymphocyte receptor (3, 4). Membrane IgD appears ontogenetically after IgM but before IgG and IgA (1), suggesting that the δ-chain gene might have evolved very early in the evolution of the immunoglobulin gene family. In order to understand the structural basis for the functional distinction of IgD from IgM, and in order to establish the evolutionary origin of the δ-chain gene in relation to other Ig heavy chain genes, it is essential to determine the amino acid sequence of IgD δ chain.

We have previously reported the isolation in large quantity of serum IgD from the plasma of myeloma patient WAH (5). Preliminary studies of WAH IgD by limited proteolysis revealed an extended hinge structure in the δ chain that contains a segment unusually enriched in electrical charge (5). This highly charged segment is responsible for the extreme sensitivity of the IgD molecule to "spontaneous degradation" (6) and is presumably involved in the T cell, B cell, and macrophage interaction during the T cell-dependent antigen triggering (4, 5). In this paper we report the complete amino acid sequence of the tryptic Fc_δ fragment [$Fc_\delta(t)$] of WAH IgD. Comparison of the WAH Fc_δ with the Fc regions of other Ig classes has enabled us to propose the evolutionary origin of the δ-chain gene. In addition, the unusually divergent sequence of the $C_\delta 3$ domain plus the unique distribution of two carbohydrate moieties on its surface strongly imply that this domain is related to the receptor function of the IgD molecule.

Our sequence for the $Fc_\delta(t)$ of WAH IgD is identical to that obtained independently and simultaneously with different procedures by Shinoda *et al.* (7) for the $Fc_\delta(t)$ of another IgD protein (NIG-65). The exchange of data, which occurred by agreement after completion of the two $Fc_\delta(t)$ sequences, provides independent verification of the results. However, the absence of interchanges in the Fc of the two δ chains does not preclude the possible existence of allotypic or isotypic differences in the Fd portion of the chains or in the Fc of other human δ chains.

EXPERIMENTAL PROCEDURES

WAH IgD was purified by a two-step procedure of ammonium sulfate precipitation plus Ultrogel AcA 34 (LKB) gel filtration. Intact IgD was cleaved by limited papain digestion into two carbohydrate-free $Fab_\delta(p)$ fragments and a $Fc_\delta(p)$ fragment that retained all the carbohydrate moieties. The $Fc_\delta(t)$ fragment used in the sequence determination was subsequently produced from the $Fc_\delta(p)$ fragment by limited tryptic digestion. Experimental details for the method have been described by Lin and Putnam (5). In addition to the $Fc_\delta(t)$ fragment, several CNBr peptides generated from the intact δ chain (CB-C_δ1-hinge-C_δ2) and the $Fc_\delta(t)$ fragment (CB-C_δ2, CB-link, and CB-C_δ3) were also used in the sequence determination of the Fc region (5, 8).

Small peptides necessary for the completion of the amino acid sequence were generated from the $Fc_\delta(t)$ fragment and the above-mentioned CNBr peptides by complete tryptic, chymotryptic, and staphylococcal protease (V8) digestion. After purification by gel filtration, ion-exchange chromatography, and paper electrophoresis/chromatography (if necessary), these peptides were subjected to amino-terminal sequence determination on a Beckman automatic sequenator (model 890C), using a 0.1 M quadrol program (no. 121078). The phenylthiohydantoin derivatives of amino acids were identified by high-performance liquid chromatography (Hewlett-Packard, model 1084A). If necessary, the carboxy-terminal sequence was determined by time-course carboxypeptidase Y digestion (9).

RESULTS AND DISCUSSION

Primary Structure of WAH $Fc_\delta(t)$ Fragment. The complete amino acid sequence of the WAH IgD $Fc_\delta(t)$ fragment is presented in Fig. 1. It is 226 residues long and includes three glucosamine-containing carbohydrate moieties: one in the $C_\delta 2$ domain and two in the $C_\delta 3$ domain. For the first 41 residues, the WAH $Fc_\delta(t)$ fragment has a sequence identical to that reported for an $Fc_\delta(t)$ fragment by Spiegelberg (10) except for a Ser/Thr interchange at residue 38. Because the latter sequence appears to be incorrect beyond residue 41, we believe this interchange

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Abbreviations: $Fab_\delta(t)$ and $Fc_\delta(t)$, tryptic Fab and Fc fragments of IgD; $Fab_\delta(p)$ and $Fc_\delta(p)$, 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. 33.

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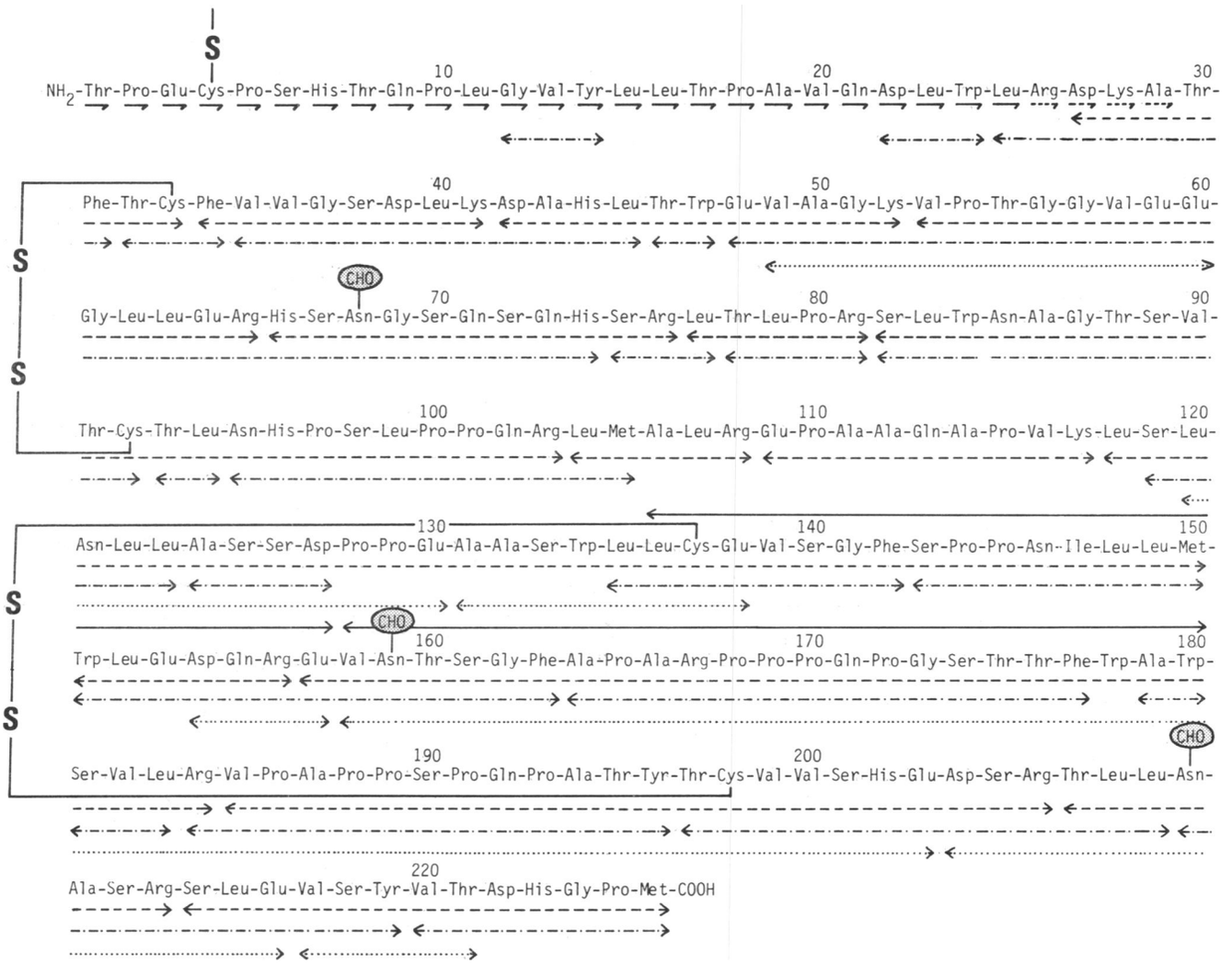

FIG. 1. **Complete amino acid sequence of the WAH IgD $Fc_\delta(t)$ fragment. Only peptides providing the necessary sequence and overlap information are underlined: ——, CNBr peptides; ---, tryptic peptides; ·····, chymotryptic peptides; ·····, staphylococcal protease (V8) peptides. Arrows under the amino-terminal residues indicate a direct sequence determination of the completely reduced and aminoethylated tryptic Fc_δ fragment. The inter-δ-chain disulfide bond is located at Cys-4; the two intradomain disulfide bonds are assigned by homology and are placed between Cys-33 and Cys-92 for the $C_\delta2$ domain and between Cys-137 and Cys-198 for the $C_\delta3$ domain. The three glucosamine-containing carbohydrate moieties (designated CHO) are attached to asparagines 68, 159, and 210, respectively.**

probably represents a technical difficulty rather than a real genetic marker.

Three methionine residues are present in the WAH $Fc_\delta(t)$ fragment. Methionines at positions 105 and 150 are responsible for the generation of the three CNBr peptides: CB-$C_\delta2$ (residues 1–105), CB-link (residues 106–150), and CB-$C_\delta3$ (residues 151–226). The latter two were not always separated into two distinct peptides because of incomplete cleavage between Met-150 and Trp-151 (8). In addition, the presence of an acid-labile bond within the CB-link peptide (between Asp-127 and Pro-128) further complicated the overall cleavage pattern (5). A third methionine residue (Met-226) occupies the carboxy terminus of the WAH δ chain, as has been suggested by Goyert *et al.* (6). However, the complete carboxy-terminal sequence was demonstrable only when intact δ chain or CB-$C_\delta3$ isolated from intact δ chain was used in the sequence analysis. This is because the carboxy-terminal segment of δ chain is extremely labile to proteolytic degradation. For example, at least three papain-susceptible bonds (after Ser-218, Thr-221, and Asp-222) and two trypsin-susceptible bonds (after Arg-206 and Arg-213) were cleaved during the preparation of $Fc_\delta(p)$ and $Fc_\delta(t)$ fragments. Nevertheless, contrary to the suggestion of Jefferis and Mathews (11), no proteolytic cleavage site was identified within the intradomain disulfide loop.

Comparison of the Fc Structures of Human Immunoglobulins. The alignment in Fig. 2 compares the amino acid sequence of the WAH IgD Fc region with the known Fc sequences of human γ1(EU), α1(Bur), ε(ND), and μ(OU) chains (18–21). The distribution of homologous residues among the five chains is not random and can be characterized into four patterns that are depicted in a spatial model for the WAH Fc region (Fig. 3). There are eight residues in the $C_\delta2$ domain and nine residues in the $C_\delta3$ domain that appear invariant among all five chains, but only three residues in each domain are absolutely invariant in all the immunoglobulin domains studied—i.e., the two cysteines forming the intradomain disulfide bridge and the tryptophan located 14–16 residues carboxy-terminal to the first cysteine. These three residues apparently form the domain nucleation center during the folding of nascent immunoglobulin chains. Residues highly conserved among all five chains are found mainly, but not necessarily, around segments occupied by the β-pleated sheets. For example, only scattered homology is

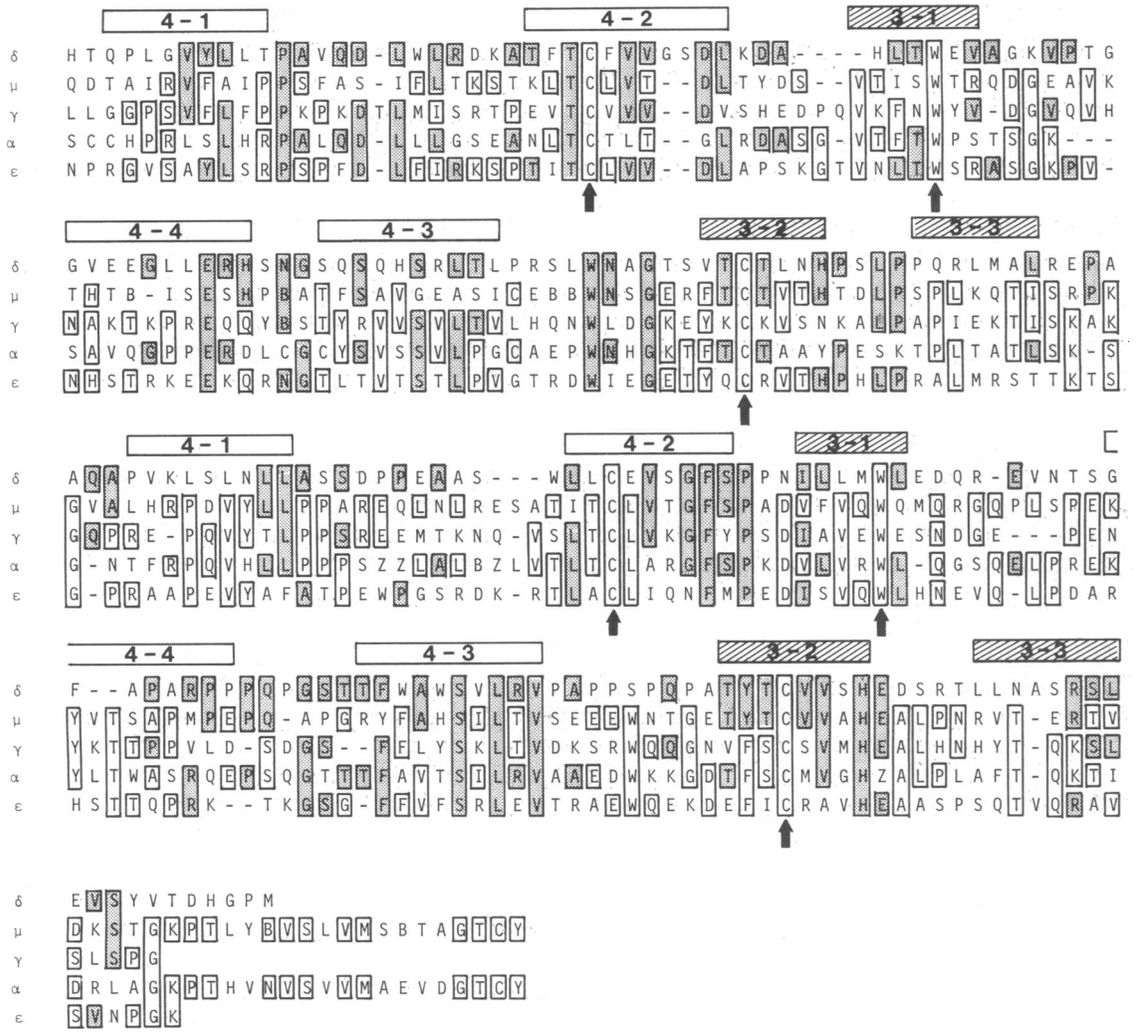

FIG. 2. Comparison of the amino acid sequences of the Fc regions of the five classes of human immunoglobulins. The one-letter notation for amino acids is given in ref. 12. The alignment is so arranged that the top two and the bottom three rows represent, respectively, amino acid residues encoded by putative exons for the last two constant domains of each heavy chain class. The boundary between the two domains is demarcated by comparison with the known junctional sequence of the mouse heavy chain genes (13–15). Gaps are introduced into the alignment to account for possible deletion/insertion events and to achieve optimal homology among all the five chains. The three invariant cysteine (C) and tryptophan (W) residues in each domain were used to help place the alignment in register, and they are indicated by arrows. Residues in the μ, γ, α, or ε sequences that share identity with the corresponding residues in the δ chain are outlined by shaded boxes; residues that show homology among sequences other than the δ chain are outlined in open boxes. The range of the β-pleated sheet structure is determined according to Beale and Feinstein (16). The β-strands are numbered according to Edmundson *et al.* (17) and are indicated above each row, with the 4-stranded β-sheet elements in open bars and the 3-stranded β-sheet elements in hatched bars.

found within strands 4-4 and 3-3 of both Fc domains (Fig. 2), presumably because these β strands occupy a marginal position in each β-pleated sheet (Fig. 3), so they are less critical in providing hydrogen bonds that are crucial to the overall domain stability. There are several peptide segments in the Fc region in which the δ-chain sequence appears to diverge from the sequences of the rest of the four chains; these include the connecting segment between the $C_\delta 2$ and the $C_\delta 3$ domains and most of the loop structure at the "back" end of the $C_\delta 3$ domain (Fig. 3). Another structural feature of the $C_\delta 3$ domain is the clustering of proline residues at both its "front" and "back" ends (Fig. 3). Many of these proline residues are unique to the δ-chain sequence, such as the five prolines found in the loop between β strands 4-3 and 3-2. Because the proline residue tends to change the course of the polypeptide backbone, such clustering of proline residues inevitably will impart to the $C_\delta 3$ domain a surface conformation significantly different from that of other carboxy-terminal domains.

The carbohydrate moiety in the $C_\delta 2$ domain is attached to Asn-68 in the β turn between strands 4-4 and 4-3 (Fig. 3). This attachment site is conserved at identical sites in the homologous domains of δ, γ, and ε chains (Fig. 2). By analogy to the observed three-dimensional structure of the IgG Fc region (22, 23), the branched polysaccharide chain from this carbohydrate moiety may not only cause a wide separation between the opposing $C_\delta 2$ domains but also prevent close contacts between the $C_\delta 2$ and the $C_\delta 3$ domains. There are two carbohydrate attachment sites in the $C_\delta 3$ domain, located at Asn-159 and Asn-210, respectively (Fig. 1); neither has a counterpart in the homologous Fc domain of other heavy chains, and thus both are unique to the IgD Fc region (Fig. 2). However, attachment sites similar to Asn-210 are found in the nonhomologous $C_\varepsilon 1$ and $C_\mu 2$ domains (20, 21). Asn-159 is located on an extended loop between the β strands 3-1 and 4-4, whereas Asn-210 is located in the carboxy-terminal β strand 3-3 (Fig. 3). Both carbohydrate moieties are spatially close to the "back" end of the Fc region, and their

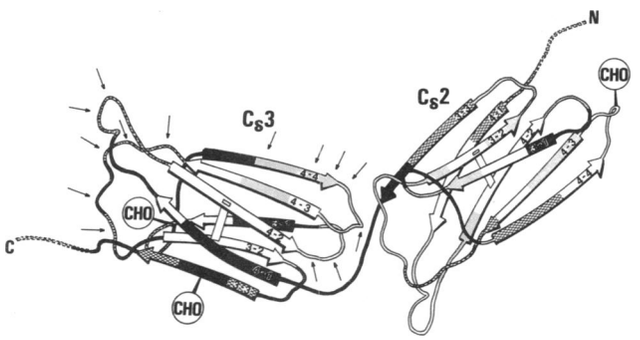

FIG. 3. Spatial model of the IgD Fc region. The schematic diagram for the α-carbon backbone of $C_\delta 2$ and $C_\delta 3$ domains is adapted from a drawing for the C_λ domain of Mcg Bence Jones dimer, for which the three-dimensional structure has been determined (17). The lengths of the β-sheet strands (broad segments) and the connecting segments are adjusted as described for Fig. 2. The shading on the backbone indicates the extent of sequence homology between the δ chain and the other four human heavy chains as follows: open, highly conserved among all five chains; shaded, scattered but significant homology among all five chains; cross-hatched, high divergence among all five chains; solid, high homology among all heavy chains except the δ chain. The three glucosamine oligosaccharides attached to the Fc region of IgD are designated CHO, enclosed in circles. Arrows pointing to the $C_\delta 3$ domain indicate the clustering of proline residues at the carboxy terminus.

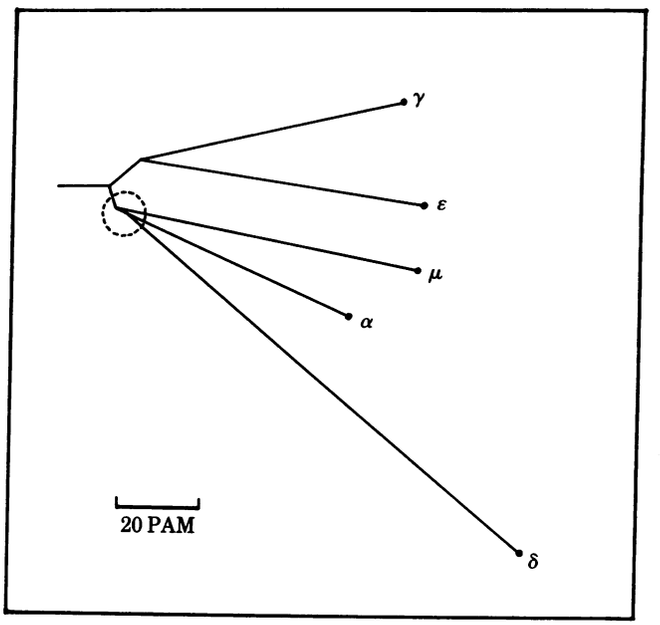

FIG. 4. Proposed evolutionary tree for human Ig heavy chains. The tree is constructed according to Barker *et al.* (26) and is based on the Fc sequences aligned in Fig. 2. The branch length is drawn proportional to the accepted point mutations per 100 residues (PAM) determined for each heavy chain class. The branching pattern enclosed within the broken circle remains to be confirmed by more thorough computer analysis.

polysaccharide chains probably spread over the outside surface of the $C_\delta 3$ domain. The carboxy termini of IgM and IgA molecules also contain carbohydrate moieties located within the tailpieces (19, 21). However, because the tailpiece is spliced off in exchange for a hydrophobic segment (24, 25), the membrane IgM (and presumably membrane IgA) will be free of carbohydrate in the last domain. Thus, the unique distribution of carbohydrate moieties in the $C_\delta 3$ domain probably is related to the biological function of IgD.

Evolutionary Origin of the δ-Chain Gene. On the basis of the homology alignment in Fig. 2, a similarity matrix of the Fc domains was compiled. This showed that the $C_\delta 2$ domain appears to be more similar to the $C_\alpha 2$ and the $C_\varepsilon 3$ domains (27.8% and 27.5%, respectively) than to the $C_\mu 3$ and the $C_\gamma 2$ domains (21.5% and 20.9%, respectively), whereas the $C_\delta 3$ domain appears to be slightly more homologous to the $C_\gamma 3$ domain (24.8%) than to the $C_\alpha 3$ and the $C_\mu 4$ domains (23.5%), and is least homologous to the $C_\varepsilon 4$ domain (20.1%). The overall homology of Fc_δ to other Fc regions is: Fc_α (25.6%) > Fc_ε (23.8%) > Fc_γ (22.8%) > Fc_μ (22.5%); this set of homology values is relatively low when compared to the values obtained from other Fc pairs. For example, the overall homology between Fc_μ and Fc_α is 34.2% (excluding the tailpiece) and between Fc_γ and Fc_ε is 33.7%. Even the less homologous Fc pair, Fc_μ and Fc_ε, has 27.5% homology. This result strongly suggests that either the δ-chain gene emerged very early in the evolution of Ig heavy chain genes or it has diverged rapidly since its emergence as a separate gene. Indeed, both features are apparent in an evolutionary tree constructed by us according to the Fc region sequences: the δ-chain gene appears to branch off the α-chain gene very shortly after the divergence of μ- and α-chain genes; in addition, the δ-chain branch exhibits a far greater mutation rate than the other four chains (Fig. 4). To date, IgD has been detected in primates (27), rodents (28–30), chicken (1), and possibly tortoise (31), suggesting that the δ-chain gene phylogenetically could be at least as ancient as the α-chain gene.

The conformity in topology of our evolutionary tree with that constructed by Dayhoff and her colleagues enabled us to incorporate the δ-chain gene into their hypothetical scheme depicting the evolutionary origin of heavy chain C-region genes (26). As shown in Fig. 5A, a domain-sized primordial heavy chain C gene (exon) duplicated internally to produce a gene with two homologous exons (step 1), which were lengthened by two partial internal duplications into a four-domain C gene (steps 2 and 3). A subsequent discrete duplication produced two identical copies (step 4); one copy obtained the carboxy-terminal tailpiece and became the μ-δ-α common ancestor, and the other copy became the γ-ε common ancestor (step 5). The γ-δ-α gene duplicated again to form the μ gene and the δ-α common ancestor (step 6). Another discrete duplication involving only the δ-α and γ-ε gene segment then completed the array of heavy-chain C region genes: μ-δ-γ-α-ε (step 7). The hinge region in the δ, γ, and α chains is presumed to have evolved independently from the extra C domain exon and its flanking introns.

An alternative pathway is depicted in Fig. 5B. After the formation of two consecutive C-gene copies (step 4), one copy underwent a discrete duplication to increase the repertoire to three (step 5). The addition of the carboxy-terminal tailpiece then committed one copy to become the μ-α common ancestor (step 6), whereas the other two copies became the common ancestors for δ-? and γ-ε, respectively. (Conceivably, if the addition of tailpiece occurred before step 5, there is a 50% chance for the δ-? common ancestor to obtain the tail-piece.) A discrete duplication of this whole DNA segment would then produce an array of six C-region genes: μ-δ-γ-α-?-ε (step 7). The DNA segment designated with a question mark in Fig. 5B specifies a hypothetical C-region gene that either remains to be discovered or was deleted in the evolutionary process.

The first pathway depicts an evolutionary tree in which the δ chain shares the same branch with the α chain (Fig. 5A), whereas the second pathway depicts a tree in which the δ-chain is on a separate branch (Fig. 5B). Although the topology of these two trees appears very different, they become indistinguishable if the duplication events all occurred very close in the evolutionary time scale. Thus, in view of the uncertainty we expe-

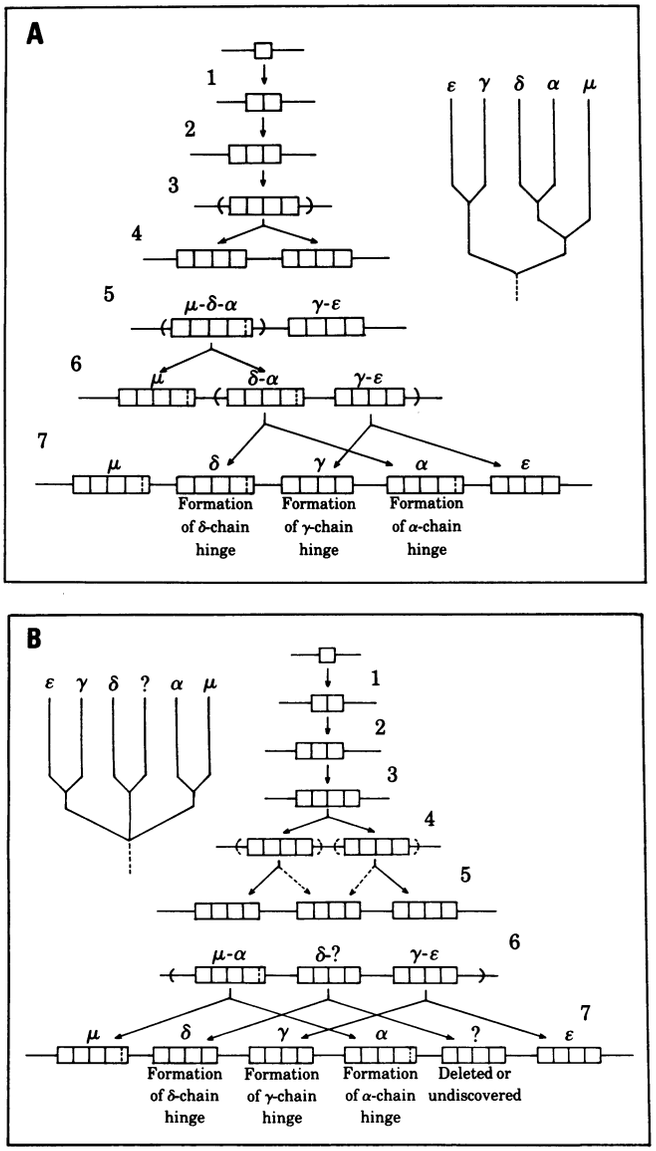

FIG. 5. Two hypothetical pathways depicting the possible genetic events that might lead to the origin of the δ-chain gene. Exons coding for the immunoglobulin C (constant) domains are boxed, with the tailpiece separated from the last C domain by a broken line. The noncoding DNA segments are represented by a thin line. For simplicity, introns between the domain exons are deleted from the diagram. The hinge region for different heavy chains is assumed to have evolved independently from the second C domain by an unknown genetic mechanism. Unbranched arrows in the pathway represent events of internal duplication that lengthened the C gene; branched arrows represent events of discrete duplication that created new C genes (parentheses on the DNA segment cover the range of discrete duplication). The evolutionary tree predicted by each pathway is shown as an inset at the top.

rienced in resolving the branching detail of our evolutionary tree (Fig. 4), we were unable to discriminate one pathway from the other. It is interesting to note that both pathways predict the order of the five known heavy-chain C genes on the chromosome as (3′) μ-δ-γ-α-ε (5′), and this gene order has recently been demonstrated in the mouse system (25, 32). Furthermore, the δ chain appears to have an additional tail that extends 6 or 7 residues beyond the carboxy-terminus of γ and ε chains, but it is much shorter than the 18- or 19-residue tailpiece of α and μ chains (Fig. 2). Although no sequence homology to the α/μ chain tailpiece is apparent, we propose that this δ-chain tail may represent a truncated relic of an ancestral tailpiece (Fig. 5).

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