

Primary structure of the immunoglobulin J chain from the mouse

(protein structure/amino acid sequence/cDNA/polymeric immunoglobulins/immunoglobulin evolution)

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ABSTRACT The primary structure of the murine J chain was investigated by sequence analysis of the J chain cDNA inserts from two independently cloned chimeric plasmids. The sequence data showed that (i) the two cDNA inserts accounted for all but approximately 100 5' nucleotides of the J chain mRNA and (ii) the J chain mRNA encodes a prepeptide of at least 23 amino acids, a mature protein of 137 residues, and an untranslated 3' region of 707 nucleotides exclusive of the 3' poly(A) tract. The amino acid sequence deduced for the mature mouse J chain was found to be 74% identical with that previously determined for the human J chain. By analyzing the conserved features of the sequence, a two-domain structure was generated for the J chain which correlates well with its functions in the polymerization of IgM and IgA. Moreover, by comparing the homologies of the J and heavy chains in mouse and man, evidence was obtained that the structures involved in polymerization are the most conserved elements of immunoglobulin molecules.

Considerable progress has been made in defining the functions of the immunoglobulin J chain. It has been found to play a critical role at two different stages of the immune response, first in the synthesis of the primary antibody product, pentamer IgM, and later in the synthesis of the major secretory antibody, polymeric IgA (1, 2). Studies of Ig polymer biosynthesis have shown that the J chain joins two monomer subunits by forming a disulfide bridge between penultimate cysteine residues in the monomer heavy chains (3, 4). In the case of IgM, the J chain-containing dimer serves as a nucleating unit to promote disulfide bonding of other IgM monomers and to complete the pentamer structure. In the case of IgA, the J chain-containing dimer is usually secreted directly from the cell, but it can induce the formation of larger polymers (5). The J chain has also been found to play a critical role in the transport of polymeric IgA to the exocrine secretions (6). Only J chain-containing polymers appear to be capable of interacting with secretory component (7), the protein that ferries the immunoglobulin across the epithelial cell wall.

In contrast, relatively little progress has been made in correlating the functions of J chain with its structure. Such studies have been hampered by the lack of mutant forms of J chain that display altered function as well as by the difficulty in isolating enough J chain for detailed biochemical characterization. Because the J chain comprises a minor fraction of the polymer protein and is highly susceptible to enzymatic degradation (8), only the human J protein has been obtained in sufficient quantities to permit sequence determination (9). Finally, it has not been possible to study the native conformation of J chain as the reducing conditions required to free the J chain from Ig polymers also reduce the intra-J chain disulfide bonds (10).

The recent development of recombinant DNA technology has provided the means to overcome some of these difficulties.

In this paper we report the primary structure of the murine J chain that was derived by sequence analysis of cloned cDNA. By comparing the data obtained with that available for the human polypeptide, the conserved features of the J chain could be identified and used to deduce structure–function relationships.

MATERIALS AND METHODS

cDNA Synthesis and Cloning. Tritium-labeled cDNA was synthesized by using as a template the poly(A)-containing RNA from the murine hybrid cell line M × W 231.1b (11, 12). After double-stranding, the cDNA was treated with nuclease S1 (Miles) to generate blunt ends (13) and was then ligated to *EcoRI* linkers (Collaborative Research) phosphorylated with [γ - 32 P]ATP by using polynucleotide kinase (New England BioLabs) (14). The *EcoRI*-adapted cDNA was size-fractionated by sedimentation through a 15–30% sucrose gradient at 4°C for 18 hr at 34,000 rpm in a Beckman SW 41 rotor. Three fractions containing the largest *EcoRI*-adapted cDNAs were treated with *EcoRI* and ligated into the single *EcoRI* site of Charon 16A (15). Phage genomes were packaged *in vitro* and the resultant phage library was plated on *Escherichia coli* K802. Recombinant phages were screened by hybridization (16) with a cloned cDNA, pJc3 (17), which was 32 P-labeled by nick-translation (18). Phages containing hybridizing cDNA were plaque-purified and the cDNA inserts were subcloned into a derivative, pBEU50, of the *ts* runaway-replication plasmid R1drd-19 (19). Plasmid amplification was achieved by shifting an exponentially growing culture of *E. coli* HB101 harboring the recombinant plasmid ($OD_{600} = 0.5$ – 1.0) from 30°C to 37°C. After 2 hr of vigorous shaking the plasmid DNA was isolated from the cells by the cleared lysate procedure (20).

DNA Sequence Analysis. The nucleotide sequence was determined by the method of Maxam and Gilbert (21) with the modified conditions for piperidine removal and electrophoresis described by Smith and Calvo (22).

Primer Extended cDNA Synthesis. cDNA was synthesized to the 5' end of J chain mRNA by using as a primer an end-labeled restriction fragment from the 5' end of Jc21. The primer was prepared by isolating the 150-base-pair (bp) *EcoRI*–*Bam*HI restriction fragment of Jc21, labeling the 5' ends with polynucleotide kinase (New England BioLabs) and [γ - 32 P]ATP, and separating the labeled ends by cleaving with *Hinf*I. Seven pmoles of the 118-bp *Hinf*I–*Bam*HI primer were hybridized to murine J chain mRNA (23) in the presence and absence of 3.5 mM methylmercury hydroxide, and the cDNA was synthesized as described above. The resulting cDNA was coelectrophoresed with DNA specifically degraded by the Maxam and Gilbert method (21) and its size was determined by using the sequence ladder as a marker.

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Abbreviations: kb, kilobase(s); bp, base pair(s).

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RESULTS

Cloning of J Chain cDNA. In previous studies a number of J chain cDNA clones were obtained with inserts ranging in size from 0.4 to 1.0 kilobase (kb) (17). Analyses of these inserts indicated that most were complementary to the 3' untranslated region of the J chain mRNA and only the largest, such as the 1.0-kb Jc3 insert, contained some coding information. Therefore, for the sequence studies reported here, it was necessary to generate additional clones by using methods that selected for longer cDNA transcripts. Of the nine J chain-positive clones recovered under these conditions, one, pJc21, was found to have an insert of $\approx 1,200$ bp. Restriction enzyme analysis showed that the Jc21 insert extended an additional 300 bp upstream from the 5' end of the previously cloned Jc3 cDNA and thus was likely to include most of the information for the 15,000-dalton J polypeptide. On this basis, the Jc3 and Jc21 clones were chosen for sequence determination.

Sequence of J Chain cDNA. The Jc3 and Jc21 cDNA inserts were subjected to sequence analysis according to the strategy diagrammed in Fig. 1, and the data obtained are given in Fig. 2. The two inserts were found to account for a total of 1,249 nucleotides. The Jc3 insert contained a terminal poly(A) stretch of 57 residues and therefore encoded the entire 3' end of the mRNA. However, the Jc21 insert did not extend to the 5' end of the message as indicated by the absence of an AUG initiation codon and an upstream untranslated region. To determine the number of 5' uncloned nucleotides, primer extension studies

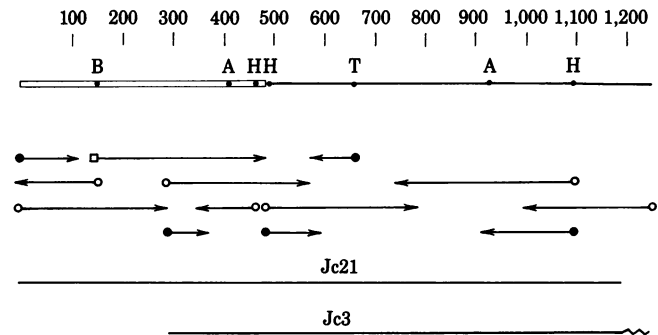
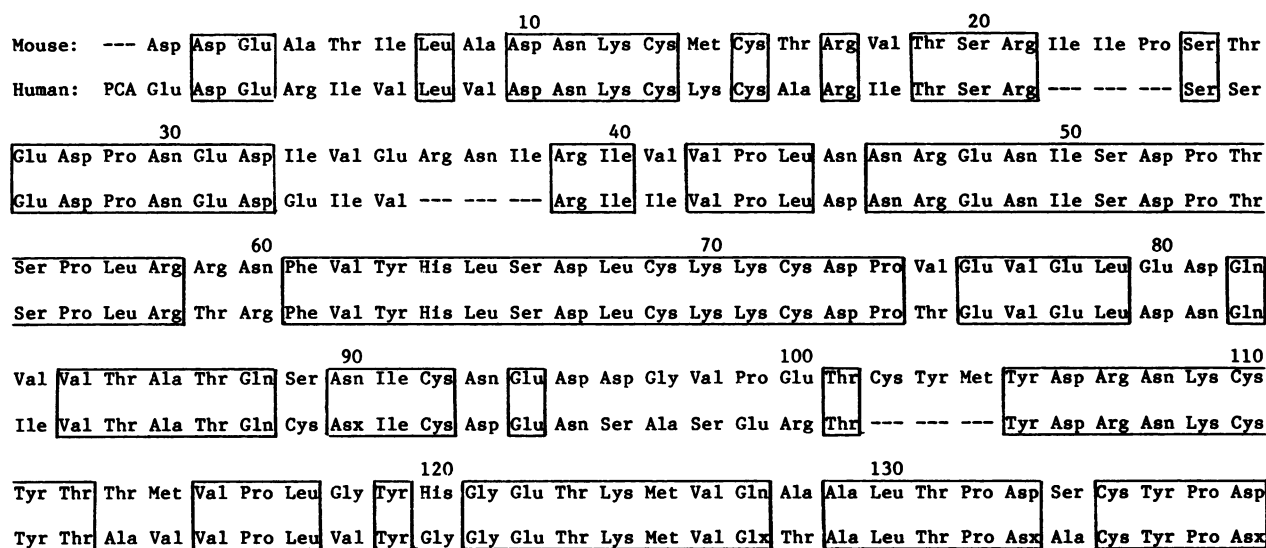


FIG. 1. Restriction map of J chain cDNA clones and the sequence analysis strategy. A map of restriction enzyme sites found in mouse J chain cDNA is displayed below a scale marked in hundreds of nucleotides; boxed area indicates coding sequences; single line, untranslated sequences; A, *Ava* II; B, *Bam*HI; H, *Hinf*I; T, *Taq* I. The Jc3 and Jc21 cDNA inserts used to derive the map are shown at the bottom of the diagram. The jagged line denotes the poly(A) tract. Closed circles represent restriction sites that were phosphorylated at their 5' ends for DNA sequence determinations; open circles, sites that were filled in at their 3' ends; open square, site that was both phosphorylated and filled in. Arrows show the direction and extent of the DNA sequence analysis reactions.

were carried out in which a 5' fragment from the Jc21 insert was used to induce synthesis of DNA complementary to the remaining 5' end of the mRNA. The cDNA transcripts generated

10	20	30	40	50	60	70	80
CA GGT GGG TCT TCA TCT TGT CTT CAC TGG GGA GTC CTA GCC ATT TTT GTT AAG GCT GTC CTT GTA ACA GGT GAC GAC GAA GCG ACC ATT							
Gly Gly Ser Ser Ser Cys Leu His Trp Gly Val Leu Ala Ile Phe Val Lys Ala Val Leu Val Thr Gly Asp Asp Glu Ala Thr Ile							
90	100	110	120	130	140	150	160
CTT GCT GAC AAC AAA TGC ATG TGT ACC CGA GTT ACC TCT AGG ATC ATC CCT TCC ACC GAG GAT CCT AAT GAG GAC ATT GTG GAG AGA AAT							
Leu Ala Asp Asn Lys Cys Met Cys Thr Arg Val Thr Ser Arg Ile Ile Pro Ser Thr Glu Asp Pro Asn Glu Asp Ile Val Glu Arg Asn							
180	190	200	210	220	230	240	250
ATC CGA ATT GTT GTC CCT TTG AAC AAC AGG GAG AAT ATC TCT GAT CCC ACC TCC CCA CTG AGA AGG AAC TTT GTA TAC CAT TTG TCA GAC							
Ile Arg Ile Val Val Pro Leu Asn Asn Arg Glu Asn Ile Ser Asp Pro Thr Ser Pro Leu Arg Arg Asn Phe Val Tyr His Leu Ser Asp							
270	280	290	300	310	320	330	340
CTC TGT AAG AAA TGC GAT CCT GTG GAA GTG GAG CTG GAA GAT CAG GTT GTT ACT GCC ACC CAG AGC AAC ATC TGC AAT GAA GAC GAT GGT							
Leu Cys Lys Lys Cys Asp Pro Val Glu Val Glu Leu Glu Asp Gln Val Val Thr Ala Thr Gln Ser Asn Ile Cys Asn Glu Asp Asp Gly							
360	370	380	390	400	410	420	430
GTT CCT GAG ACC TGC TAC ATG TAT GAC AGA AAC AAG TGC TAT ACC ACT ATG GTC CCA CCT GGG TAT CAT GGT GAG ACC AAA ATG GTG CAA							
Val Pro Glu Thr Cys Tyr Met Tyr Asp Arg Asn Lys Cys Tyr Thr Thr Met Val Pro Leu Gly Tyr His Gly Glu Thr Lys Met Val Gln							
450	460	470	480	490	500	510	520
GCA GCC TTG ACC CCC GAT TCT TGC TAC CCT GAC TAG ttgattcactcaccatgagctcgtgttccttagaggctctccatttgcaccagaagttatactcgtctgt							
Ala Ala Leu Thr Pro Asp Ser Cys Tyr Pro Asp amber							
560	570	580	590	600	610	620	630
aatgaatttgaac aggggtttttttccctctgtgtataaaactaatgttccctttcaatcattagaatatcagaattgctgtcgtgtgtgtgttaactctcaattcgaagtcgtaa							
680	690	700	710	720	730	740	750
atcctcatgtctgtcaagggtgtgttttaaagtattttccagtgttttaaaattgtaagcaagcgggaaagtaaaattccaggagtaaaagtcaagaggttaataaaccacac							
800	810	820	830	840	850	860	870
cttttcttcttcttcttctgtgtgagagctggagcttttcgcacatcctgtactagtctttctcttaacctctcactgtgttagagaaatcgccaatgaacacaggaagttacgtatctt							
920	930	940	950	960	970	980	990
cactagaagtttcaaggacctgttttggaaatatttactaatatttatgaaagactttgttgaagtgatataatttgatgcacaaatgaaaaaaatggatgttgatataatatata							
1000	1010	1020	1030	1040	1050	1060	1070
gacttgccattagatttcttggataatattttgacagtgagattttatctgaaattcttaagggagctcttgatgtctcagccaaatcgaatttgaatttgcataaatatgta							
1080	1090	1100	1110	1120	1130	1140	1150
ttctctagtacagtttgaacaattaaatagagtgctaaagcatA56							

FIG. 2. Nucleotide sequence of murine J chain mRNA. Except for ≈ 100 uncloned nucleotides at the 5' end of the mRNA, the entire nucleotide sequence of the mouse J chain mRNA is shown. Sequences encoding the mature protein and part of the leader peptide are displayed in capital letters arranged in triplets with the derived amino acid listed below each triplet. Untranslated sequences are shown in lowercase letters, terminating in a poly(A) tract.



were found to extend 98 ± 10 bases ($\pm$ SEM) beyond the 5' terminus of the Jc21 cDNA sequence. From this information it was calculated that the mature J chain message consists of $1,290 \pm 10$ nucleotides ($\pm$ SEM) plus a poly(A) tract of 57 or more residues. These data are consistent with the size of the message as determined by gel electrophoresis (17), provided a correction is made for the drag effect of the poly(A) tail on the mobility of the RNA (R. Perry, personal communication).

The amino acid sequence deduced for the murine J chain is presented along with the nucleotide sequence in Fig. 2. The murine J chain mRNA was found to encode at least 160 amino acids, more than that expected for the mature J protein. By comparing the mouse data with the amino acid sequence determined for the human J protein (9), the extra amino acids appeared to comprise part of an amino-terminal signal peptide. The precise boundary between the signal peptide and the mature J peptide could not be definitely assigned because the amino-terminal residue of the mouse J chain has yet to be identified. However, by analogy to the human sequence (Fig. 3), it seems likely that the signal peptidase cleaves between a glycyl and an aspartyl residue to yield a signal peptide of at least 23 amino acids and a mature J chain of 137 amino acids. Cleavage at this point would mean that Jc21 cDNA contains most of the prepeptide information but lacks a few 5' codons including the initial AUG. Cleavage at this point would also imply that the mouse J chain has an unblocked amino-terminal residue in contrast to the cyclized glutamic acid found in the human J chain (9).

As predicted from its size (17), the mouse J chain mRNA was found to have a large 3' untranslated region, extending 707 nucleotides. This length falls within the upper range of the values reported for the 3' untranslated regions of other eukaryotic mRNAs (24). A single polyadenylation signal was found to be present ≈ 20 nucleotides 5' from the poly(A) tract. Rather than the consensus hexanucleotide, A-A-U-A-A-A, the signal here appears to be the variant heptanucleotide, A-A-U-U-A-A-A, which has also been identified in rat amylase mRNA (24) and anglerfish pancreatic somatostatin mRNA (25).

Homology Between Mouse and Human J Chains. The amino acid sequences of the mature mouse and human J chains are compared in Fig. 3. Because the mouse J chain was found to contain eight more amino acid residues than the human protein, three gaps were inserted in the human J chain sequence to

maximize the homology. With such an alignment the two proteins exhibited 74% identity. This value probably represents a low estimate of the homology because of uncertainties in the data for the human protein. For example, the extra tripeptide comprising residues 22–25 of the mouse J chain occurs at an alignment junction in the human sequence data that was not confirmed by analysis of an overlapping peptide, and the non-homologous stretch from residues 95–100 may reflect the fact that much of the human sequence in this region was deduced from amino acid composition data. However, even the observed

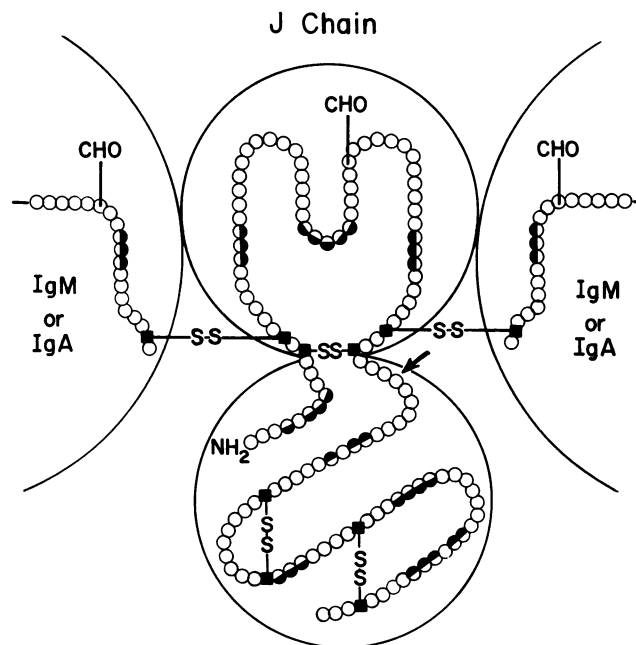


FIG. 4. A proposed two-domain structure for J chain. A model for the three-dimensional structure of mouse J chain is schematically represented by using half-closed circles to indicate stretches of hydrophobic amino acid residues, closed boxes to denote cystine residues, and open circles to represent all the remaining amino acid residues. The arrow indicates a peptide bond hypersensitive to cleavage by subtilisin. Also depicted are the carboxyl-terminal IgM or IgA domain and the 19-residue secreted tails to which the J chain is disulfide-bonded. See text for more detailed explanation.

value of 74% homology indicates that the J chain has been highly conserved over evolutionary time. In particular, features important to the three-dimensional structure have been maintained. Thus, both the mouse and the human J chains contain eight cysteine residues, seven of which occupy identical positions in the sequence, and both polypeptides display stretches of distinctly hydrophobic residues that alternate in the sequence with stretches of more hydrophilic character (see Fig. 4).

These findings are consistent with other evidence for J chain conservation. *In vitro* polymerization studies have shown that the human J chain can substitute for the mouse J chain in promoting the assembly of mouse pentamer IgM (unpublished observations). Immunological analyses have shown that the human J chain shares determinants with the J protein from descendants of primitive vertebrates, such as the dogfish and leopard sharks (2).

DISCUSSION

By combining the sequence data presented in this paper with the information available on J chain cystine bridges, it was possible to deduce a two-domain structure for the J chain that is shown schematically in Fig. 4. Analysis of the sequence data revealed that the two halves of the J polypeptide have very different characteristics. The sequence in the amino-terminal half exhibits a mirror-image symmetry that is bounded by the two sets of cysteine residues and includes the intervening stretches of hydrophobic and hydrophilic residues. In contrast, the sequence in the carboxyl-terminal half shows no apparent symmetry except for the repeating Cys-Tyr sequence. Analyses of the disulfide content of the polymeric immunoglobulins have shown that all the J chain cysteines are linked in intra- or interchain bonds (3). Moreover, cysteine-15 has been implicated in one of the interchain bonds; a tripeptide with a sequence identical to that at positions 15–17 of the human J chain was found to be linked to the penultimate cysteine residue in human polymeric IgA (26). These considerations of primary and tertiary structure suggested to us that the amino-terminal half of the J chain is folded so that an internal disulfide bond is formed between two of the distant cysteine residues. The result would be a symmetrically arranged domain with one cysteine of each set (including cysteine-15) available for intermonomer linkage. On the other hand, the features of the carboxyl-terminal half of the J chain suggested that the folding is less regular and allows the cysteines to form internal bonds spanning relatively short stretches of sequence. This would create a second domain of distinctly different structure.

A two-domain model is consistent with predictions of J chain secondary structure. Analyses of the sequence by the method of Chou and Fasman (27) indicate that the proposed domains have very different conformational properties. The sequence within the amino-terminal disulfide loop was calculated to have a high potential for forming three β pleat segments (P_β for residues 15–23, 37–44, and 61–69 = 1.23, 1.28, and 1.17, respectively) connected by stretches of random coil. Such a conformation would facilitate the interaction of this domain with the β -pleated structure assigned to the carboxyl-terminal domain of IgM or IgA (28). In contrast, the second J chain domain is predicted to have three helical segments (P_α for residues 4–10, 74–85, and 122–130 = 1.21, 1.20, and 1.24, respectively). The intervening sequence, residues 87–120, is computed to be random coil interrupted by two short β pleat segments.

The proposed two-domain structure is also supported by data on J chain proteolysis. The J polypeptide in human or murine pentamer IgM is highly susceptible to digestion with subtilisin, quantitative cleavage being achieved after brief treatment at

very low enzyme to IgM ratios (8). Analysis of the digested J chain has shown that the initial cleavage occurs in an exposed region midway in the sequence. The amino-terminal half of the J chain remains associated with the pentamer and resists degradation, whereas the carboxyl-terminal half is released and then digested into smaller peptides. This cleavage pattern confirms two assumptions of the proposed structure—namely, that both J-monomer bonds are located in the amino-terminal half of the molecule and the amino-terminal, but not the carboxyl-terminal, portion of the J chain is in close contact with the $C\mu 4$ domains of the IgM subunits. Analyses of the digested pentamer have indicated that its three-dimensional structure remains intact; no changes could be detected by electrophoretic or ultracentrifuge measurements in various solvents (8, 29). Moreover, the digested pentamer retains most of its capacity to fix complement and bind secretory component (29). Thus, it would appear that the polymerizing function of the J chain resides in its amino-terminal half, whereas the function of the carboxyl-terminal half of the molecule remains to be determined.

It should be emphasized that the model shown in Fig. 4 represents a tentative structure of J chain. Although the proposed two-domain arrangement is substantiated by experimental data, many of the intradomain features—e.g., the assignment of intrachain bonds and the interaction of hydrophobic regions—remain purely hypothetical. It may be possible to ascertain the location of the J chain disulfide bridges by using chemical cleavage methods (9), but the resolution of other features of the J chain structure will require analysis of the three-dimensional structure of the parent polymers. Until such studies are accomplished, the proposed model can serve as a useful framework for probing the structure–function relationships of the J chain.

In addition to providing insight into the structure of J chain, the sequence data reported here made it possible to assess the conservation of both the J chain and the polymerization process.

Table 1. Homology of the J chain domains and the heavy chain constant region domains from man and mouse

Comparison of human vs. mouse	Domain				COOH terminus*
	1	2	3	4	
J chain domain	79% (57/63)	69% (71/75)	—	—	—
μ chain constant domain	48% (106/106)	59% (106/106)	53% (107/107)	78% (111/111)	89% (19/19)
α chain constant domain	42% (98/98)	64% (102/102)	78% (77/77)	—	68% (19/19)
γ chain constant domain†	62% (48/92)	66% (110/110)	57% (103/103)	—	—

In each domain column the ratio in the parenthesis shows the number of amino acids compared in the homology determination relative to the total number of amino acids in that homology unit; the percentage value is the percent homology that was calculated by determining the number of sequence identities among the amino acids compared. Sequence gaps (insertions or deletions) were not counted in the homology calculations.

* COOH terminus, carboxyl-terminal sequences of secreted μ and α chains.

† Human C γ 1 vs. mouse C γ 2a; comparisons of the homology between human C γ 4 and mouse C γ 3, human C γ 2 and mouse C γ 1, and human C γ 3 and mouse C γ 2b gave similar results.

When the sequences of the mouse and human J chains were compared, the two proteins were found to be highly homologous, ranging from 79% identity in the amino-terminal "domain" to 69% in the carboxyl-terminal domain (Table 1). Similar homologies have been observed for the regions of the IgM and IgA monomers that are involved in the polymerization process. These include the carboxyl-terminal constant domains and the adjoining 19-residue tails that are unique to secreted μ and α chains and contain the penultimate cysteine residue through which polymerization is effected (1, 2). Kehry *et al.* (30) have found that the carboxyl-terminal regions of μ chains are considerably more conserved than the amino-terminal regions, and similar results have recently been obtained for α chains (31). The mouse and human data from these studies are presented in Table 1 along with representative data for the IgG subclasses (32, 33) which do not undergo polymerization. It can be seen that the C μ 4 and C α 3 domains of the mouse are 78% homologous with the human C μ 4 and C α 3, a value very similar to that obtained for the J chains, and the homologies of the respective carboxyl-terminal tails fall within the same range. In contrast, the sequences of other C μ and C α domains and the sequences of all the C γ domains, including the carboxyl-terminal, have diverged to a significantly greater extent. These comparisons indicate that the structural requirements for polymerization have imposed such strong selective constraints on the J chain and the carboxyl-terminal regions of the μ and α chains that these elements are the most conserved in the immunoglobulin system.

The question then arises as to why the polymerization process has been maintained over the evolution of the immunoglobulins. Although pentamer IgM was the first functional antibody to appear in the primitive vertebrates, monomeric forms with generally higher antigen affinity and specificity have subsequently evolved. Moreover, the organization of the heavy chain gene locus indicates that the synthesis of the secreted forms of μ and α chains could be bypassed (34). One possible explanation for the conservation is that the polymeric antibodies play an essential role in protection against disease, pentamer IgM by acting at the early stages of infection and polymeric IgA by protecting the body surfaces from colonization by pathogens. Because of their multivalence, the polymeric antibodies could effectively complex those pathogens displaying repeating sets of determinants on their surface. A second possible explanation is that the polymerization of IgM is an obligatory step in the differentiation of the antibody-producing cell. Some component of the polymerizing system, pentamer IgM itself, or a by-product generated during its synthesis, may be required to signal the switch in immunoglobulin class synthesis that follows the pentamer IgM response. By pursuing the analysis of the polymerizing system at the molecular level, it should be possible to resolve the question of its function and thus of its high degree of conservation.

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