The Switch Point in μ Heavy Chains of Human IgM Immunoglobulins†

Gerard Florent,‡ David Lehman, and Frank W. Putnam*

ABSTRACT: Comparative structural study of four IgM macroglobulins from different patients has been done to determine the switch point between the V (variable) and the C (constant) regions of μ heavy chains and to aid in elucidating the mechanism governing their genetic control. Three new μ chains were partially sequenced, Ga, Di, and Dau; these were compared with the μ chain of IgM Ou for which the complete amino acid sequence has been reported. Although many differences were found in the V region of the four μ chains, no differences have yet been identified in the C region. The C region begins at residue Ser-125 (in the Ou numbering system) and in each μ chain is preceded by a variable region that has a unique amino acid sequence. The V region sequence is independent of the class of the heavy chain, and subgroups denoted V_{HI}, V_{HII}, and V_{HIII} have been proposed which are shared by all classes of heavy chains (μ, γ, α, δ, and ϵ). Extensive sequence analysis of the four μ chains generally supports this classification, but the subgroups appear less well defined than previously thought. The division of μ chains into V and C regions supports the hypothesis that separate V region and C region genes are required for biosynthesis of heavy chains.

Antibodies are immunoglobulin molecules consisting of a pair of heavy chains and a pair of light chains disulfide bonded to each other in a symmetrical tetrapolypeptide chain structure that has two equivalent combining sites for specific antigen. For κ and λ light chains, and for the γ heavy chain, it has been shown that each chain is divided into a variable (V) region comprising the first 110–120 amino acid residues of the amino terminus and a constant (C) region representing the rest of the chain. It is believed that all immunoglobulin polypeptide chains have this basic structure and that the C region has a characteristic amino acid sequence which defines the type (κ or λ) of light chain or the class (γ, α, μ, δ, or ϵ) of heavy chain. The V region differs idiotypically, that is individually for each patient with a plasma cell tumor, such as multiple myeloma or macroglobulinemia, and is believed to differ uniquely for each antibody specificity (Putnam, 1969). A principal problem of molecular immunology today is to establish the structural characteristics of each class of heavy chain as an aid to the elucidation of the genetic control of structural variability in immunoglobulins and its relationship to antibody specificity. To this end, we have determined the complete amino acid sequence of one μ chain and the partial amino acid sequence of three other μ heavy chains from human IgM immunoglobulins; these results have enabled definition of the switch point between the V and C regions of μ chains and identification of conservative and hypervariable segments with different functions in the V region.

The experimental design of this work was to dissect out large accessible fragments of IgM proteins Ga, Di, and Dau that were suitable for analysis with the sequenator and to compare the sequence of these fragments with the complete amino acid sequence established for the Ou μ chain by a combination of conventional and automatic methods (Putnam *et al.*, 1973). Since all four μ chains are blocked, direct analysis of the N-terminal region with the sequenator was not possible. One of the main objectives was to isolate a large fragment containing the switch region, that is, the segment in which the sequence changes over from variable to constant. From previous work we had postulated that the Cμ region begins with Val-123 in the sequence Val-Ser-Ser (Putnam *et al.*, 1971, 1972). If our scheme for the structure of the μ chain is correct, the first methionine in the C region should be Met-204 in the Ou numbering system.[1] Because the location and number of methionine residues in the V region differ for different μ chains, the number of CNBr fragments obtained from the V region will vary. Furthermore, although the sequence of the switch region should be constant from the V/C juncture to Met-204, the amino terminal portion of the switch region will be variable, as will the length of the fragment. To obtain the switch region peptide, Fab fragments of IgM were prepared by tryptic cleavage at 60°; these were succinylated, cleaved with CNBr, reduced, and alkylated, and the resulting fragments were separated by gel filtration and were sequenced.

Experimental Section

Materials. Plasma containing IgM in high concentration (2–8 g/100 ml) was obtained from four patients with Waldenström's macroglobulinemia, designated Ou, Ga, Di, and Dau.[2] Sephadex products were obtained from Pharmacia

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‡ Present address: Recherches et Industries Therapeutiques, Brussels, Belgium.

[1] Because μ heavy chains may have different lengths in the V region and because the complete sequence was not determined for IgM proteins Ga, Dau, and Di, the numbering of residues for all chains is referred to the numbering system for the Ou μ chain (Putnam *et al.*, 1973) in comparisons of the sequences. However, in presentation of the sequence for Ga, for which the entire V region was determined, a continuous numbering system beginning with the N-terminus is used.

[2] Plasma was obtained from cooperating physicians as follows: Ou, Dr. John Fahey and Dr. William D. Terry, National Cancer Institute, Bethesda, Md.; Ga, Dr. Samuel Murphy, Ohio State University School of Medicine, Columbus, Ohio; Di, Dr. Thomas Newcomb, University of Florida School of Medicine, Gainesville, Fla.; Dau, Dr. John Harter, Massachusetts General Hospital, Boston, Mass. Antiserum for normal IgM and antiserum prepared with IgM Ga as an antigen were supplied by Dr. Fred Hymes of Meloy Laboratories, Inc. Large scale purification of IgM Ga was done for us by Drs. Milan Wickerhauser and Yu-lee Hao of the American National Red Cross Blood Research Laboratory, Bethesda, Md.

Fine Chemicals, and carboxypeptidases A and B and Tos-PheCH$_2$Cl-trypsin from Worthington. Reagents used for the sequenator (sequenal grade) were from Beckman Instruments Inc. Reagents used for amino acid analysis and sequence determination have been described (Titani *et al.*, 1970). Antisera were prepared by immunization of rabbits with purified IgM, μ chains, or light chains (Migita and Putnam, 1963) or were obtained from Meloy Laboratories, Inc.[2]

Methods. Preparation of IgM. The 19S IgM was isolated from the plasma by a combination of euglobulin precipitation and gel filtration based on the procedure of Putnam *et al.* (1967).[3] The plasma was first dialyzed against a solution of 0.1% $CaCl_2$ to remove the fibrinogen. After a tenfold dilution with distilled water, the plasma was dialyzed in the cold against distilled water to precipitate the IgM. After centrifugation, the IgM globulin was purified by gel filtration on Sephadex G-200 to remove any contaminating IgG. The immunochemical purity of the IgM preparations was checked qualitatively by immunoelectrophoresis and semiquantitatively by immunodiffusion using serial dilutions with antisera that were monospecific for IgM, IgA, IgG, and for various plasma proteins; by these criteria and by analytical ultracentrifugation, the IgM preparations were at least 98% pure and contained less than 1% IgG. Of course, in analytical ultracentrifugation minor IgM peaks representing higher polymers of IgM, such as 22S and 26S components, were present in addition to the predominant 19S component. All of the four IgM globulins had κ light chains. No subclassification of the IgM proteins could be made by methods using various antisera specific for the μ chain or for the Fc region.

Preparation of Fabμ Fragment. The Fabμ fragment consists of the light chain attached by a disulfide bond to the Fd′ portion (about 213 residues) of the μ heavy chain characteristic of IgM. For all four IgM proteins the Fabμ fragment was prepared by the "hot trypsin" method; that is, by limited cleavage of undenatured IgM by incubation at 60° with trypsin for 45 min with a ratio of enzyme to substrate of 1:50 and an IgM concentration 1.5% (Plaut and Tomasi, 1970). The Fabμ fragment (s_{20} = 3.5 S, molecular weight of about 50,000) was separated from the Fcμ fragment (s_{20} = 10 S, molecular weight of about 340,000), Fcμ is a decamer of the carboxyl-terminal portion of the μ heavy chain. Sometimes uncleaved IgM remains associated with the Fcμ fragment in the column eluate. The third and last peak eluted consists of a mixture of peptides resulting from extensive cleavage of some molecules and from the excision and degradation of the third homology region of the μ chain (designated Cμ2 because it is the second homology region of the constant portion of the μ chain). With different proteins and in different experiments with the same protein, the yields varied somewhat, but the usual recovery of Fabμ was about 40% of theoretical.

Succinylation of Fabμ. In order to obtain larger peptides suitable for sequenator analysis the IgM proteins were succinylated. This blocks the lysyl groups and also improves the solubility of the reduced–alkylated μ chains and of the CNBr fragments. The IgM proteins were succinylated with solid succinic anhydride which was added in small portions to the protein solution over a period of 1 hr in a ratio of 5 g of succinic anhydride per g of protein. The pH was kept between 8 and 10 by adding solid Na_2CO_3. The excess of reagent was removed by dialysis (Klotz, 1967).

The cyanogen bromide cleavage was performed on succinylated Fabμ in 70% formic acid by reaction for 4 hr at room temperature at a ratio of 5 g of CNBr/g of protein. The excess of CNBr was removed by lyophilization (Witkop, 1968).

Reduction and Alkylation. After succinylation of the Fabμ and reaction with CNBr, all disulfide bridges were reduced and alkylated by reduction with 0.1 M mercaptoethanol in 8 M urea buffered with 0.1 M Tris-HCl (pH 8.0), followed by alkylation with 0.2 M iodoacetamide (Titani *et al.*, 1969).

Enzymatic Hydrolysis. Tryptic hydrolysis of the reduced–alkylated, succinylated CNBr fragments was done by incubation at 37° for 30 min in 1% ammonium bicarbonate with a 1:50 weight ratio of trypsin to substrate. Chymotryptic hydrolysis was done under the same conditions. Purification of the peptides is described in the Results section.

Amino Acid Analysis. Samples for amino acid analysis were hydrolyzed for 22 hr with 5.7 N HCl in an evacuated sealed tube and analyzed with the Beckman Model 120 automatic amino acid analyzer equipped with high sensitivity cuvets and recorders. Glucosamine was determined from the short column analyses; no correction was made for destruction by acid hydrolysis, which is considerable (Moore and Putnam, 1973).

Amino Acid Sequence Analysis. Sequence analysis was done almost entirely by the automatic method with the Beckman Model 890 sequencer. The phenylthiohydantoins were identified by gas chromatography with two columns (Pisano and Bronzent, 1969) with verification of the regenerated amino acid as needed. For automatic sequence analysis, procedures similar to those of Hermodson *et al.* (1972) were followed. The amino end groups of peptides were determined by the dansyl-Edman method (Gray, 1967), and the dansyl derivatives were characterized by the procedure of Woods and Wang (1967). Information on the carboxyl-terminal sequence of peptides was obtained by incubation for 1 hr at 37° in 0.1 M Tris-HCl buffer (pH 8.0) with a mixture of carboxypeptidases A and B. The liberated C-terminal residues were determined with the amino acid analyzer.

Nomenclature of the Peptides. Because the tryptic peptides were prepared from succinylated CNBr fragments of 4 μ chains having different amino acid sequences in the V region, a nomenclature system was devised which identifies each peptide with the μ chain and the CNBr fragment from which it was derived. In this system, V and C represent the variable and constant regions, respectively. This is followed by designation for the μ chain referring to the patient from whom the IgM was obtained, *e.g.*, Ou, Ga, Di, or Dau. Cyanogen bromide fragments are indicated by the symbol B followed by a number indicating the order of this fragment in the μ chain from which it is derived. Tryptic peptides from a succinylated CNBr fragment are designated T followed by a number indicating their order in the fragment. For example, VDiB1T1 indicates the amino-terminal tryptic peptide T1, which is derived from the amino-terminal CNBr fragment VDiB1 which is in the V region of the μ chain from IgM Di. If lysyl peptides were prepared from the unsuccinylated μ chain, a second number is given to indicate the order within the succinylated tryptic peptide, *e.g.*, the succinylated tryptic peptide from the amino terminus of the Di μ chain is VDiB1T1, and the corresponding peptides from the unsuccinylated μ chain are VDiB1T1-1 and VDiB1T1-2. Because the V regions of μ chains vary in length and also because the V region

[3] We are indebted to Dr. Yu-lee Hao and Dr. Milan Wickerhauser of the American National Red Cross Research Center, Bethesda, Md., for collaboration in the large scale purification of the plasma from patient Ga and for a supply of partially purified, normal human IgM.

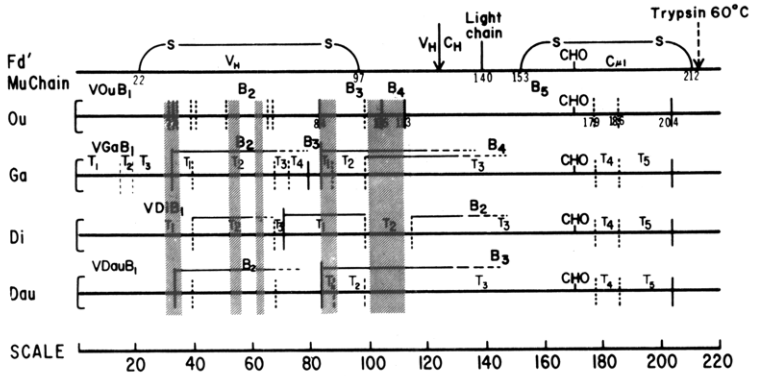

FIGURE 1: Schematic alignment of the CNBr fragments of the Fd portion of the μ heavy chain of human IgM immunoglobulins Ou, Ga, Di, and Dau. The fragments are aligned with reference to the known amino acid sequence of the Ou μ chain (Putnam *et al.*, 1973). The position of methionine residues is indicated by vertical solid lines and of arginine residues by vertical dotted lines. The bracket at the N terminus indicates the chain has a blocked end group of pyrrolidonecarboxylic acid. The sequence of each chain is depicted by the heavy horizontal line to the scale given below. Light horizontal lines for Ga, Di, and Dau indicate analyses with the sequencer. Tryptic peptides from the succinylated chains are designated T1, T2, etc. CNBr fragments are denoted B1, B2, etc., as described in the text. CHO indicates carbohydrate. Vertical shaded lines identify regions of sequences that are hypervariable in the V region of all heavy chains.

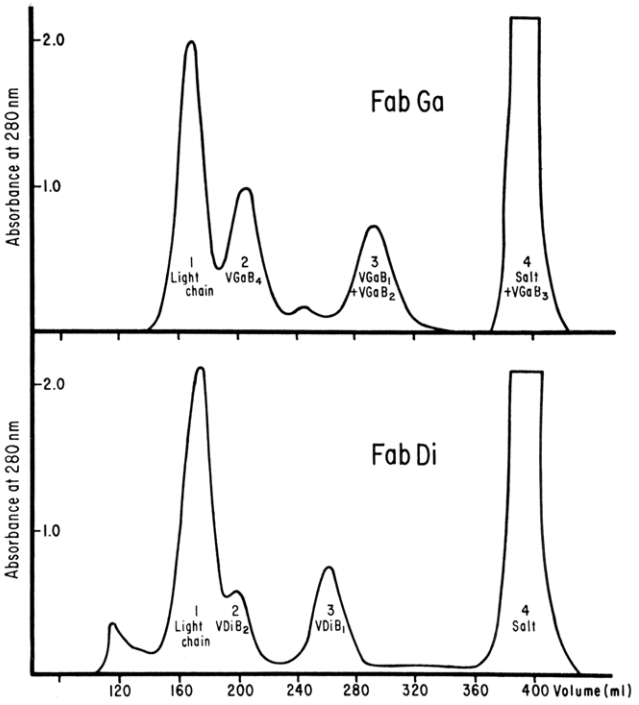

FIGURE 2: Separation of CNBr fragments of succinylated, reduced, and alkylated Fab fragments of IgM immunoglobulins Ga (upper diagram) and Di (lower diagram). Gel filtration on Sephadex G-100 was done at room temperature with 6 M urea, 0.05 M formic acid; the column size was 2.5 × 100 cm and the fraction size was 3.5 ml.

sequences were not complete for all four μ chains, the numbering system for amino acid residues is based on homology of the sequence to that of the V region of the Ou μ chain, for which the complete sequence has been reported (Putnam *et al.*, 1973). To facilitate identification of the CNBr fragments and the tryptic peptides described in the Results section, a schematic diagram is given in advance in Figure 1 showing their localization in the various μ chains. Evidence for this localization is given in the Results and Discussion sections and in later figures.

Results

Isolation of CNBr Fragments. The polypeptides obtained by CNBr cleavage of the reduced–alkylated, succinylated Fabμ fragments of IgM Ga, Di, and Dau were separated by gel filtration on Sephadex G-100 in 6 M urea–0.05 M formic acid at room temperature. Figure 2 shows the elution pattern obtained for the Fabμ fragments of Ga (upper figure) and Di (lower figure). A somewhat different elution pattern was obtained for the Fabμ fragment of IgM Dau because the κ light chain also contains methionine and yields two CNBr fragments.[4]

Because of the varying number and distribution of the methionine residues in the V regions of μ chains and the possibility of methionine residues in light chains the elution profiles may differ significantly for different Fabμ fragments.[5]

In each case in Figure 2 the first major fraction contains light chain and is devoid of methionine and homoserine. The second fraction (VGaB4 for Fabμ Ga, and VDiB2 for Fabμ Di) is a large fragment that includes the V/C switch area. This was first suggested by the presence of a large amount of glucosamine attributable to the carbohydrate at position 170 in the μ chain, for carbohydrate has been identified at this position in the Ou and Di μ chains (Shimizu *et al.*, 1971; Putnam *et al.*, 1972). Carbohydrate is located in a similar sequence in the Dau μ chain according to unpublished results of our laboratory and preliminary data of Hurst *et al.* (1973). Accordingly, the third peak should contain the CNBr fragment that is N-terminal in the Fd portion of each μ chain. In the separation for Fabμ Ga the third peak contains two CNBr fragments (VGaB1 and VGaB2), each of about 40 residues in length, whereas only one fragment (VDiB1) was present in the third peak for Fabμ Di. In addition, a CNBr fragment that was only a tetrapeptide (VGaB3) was found in the salt peak for Fabμ Ga. The position of the third fraction eluted from the CNBr cleaved Fabμ Di indicates that the fragment (VDiB1) is larger than the fragments (VGaB1 and VGaB2) in the corresponding fraction for Fabμ Ga. In the case of Di the resolution between the light chain and the fragment including the switch area (VDiB2) was poor. The resolution of VGaB1 and VGaB2 was accomplished by gel filtration of the CNBr cleavage products of Fabμ before and after reduction–alkylation, as described below.

The amino acid composition, NH_2-terminal residues, and probable placement of the CNBr fragments of the Fd' region of the Ga μ chain (VGaB1 to VGaB4) and of the Di μ chain (VDiB1 and VDiB2) are given in Table I. The carboxyl-terminal amino acids of some of these fragments are given in Table II. The pattern of the separations, the nature of the N-terminal residues identified in each fraction (Table I),

[4] Because extensive structural studies of IgM Dau will be reported separately (Lehman, D., Ph.D. Dissertation, in preparation), detailed data on the preparation and sequencing of the CNBr fragments are not given in this paper.

[5] Amino acid analyses of the Fabμ fragments are not reported because the presence of a different κ light chain in each Fabμ fragment greatly affects the results. Predicted numbers of CNBr fragments are not given because the number of methionine residues in the V region of each μ chain has to be estimated by difference and exact stoichiometry is difficult to obtain for very large proteins such as IgM. Thus, the numbering of CNBr fragments is based on the number actually isolated, and their location is based on sequence determination and homology to the Ou μ chain.

TABLE I: Amino Acid Composition of the CNBr Fragments from VGa and VDi.[a]

	VGaB1	VGaB2	VGaB3	VGaB4	VDiB1	VDiB2
Lysine		2.6 (3)		4.3 (4)	4.1	3.6 (4)
Histidine		1.0 (1)				
Arginine	2.3 (2)	2.8 (3)		4.0 (4)	3.5	5.0 (4)
CM-cysteine	+(1)			2.4 (3)	0.5	2.0 (3)
Aspartic acid		4.8 (6)		11.0 (11)	4.8	10.6 (11)
Threonine	1.3 (1)	1.9 (2)		8.5 (9)	4.0	12.4 (10)
Serine	5.2 (6)	4.4 (5)		15.0 (19)	5.8	20.2 (22)
Homoserine	0.8 (1)	0.5 (1)	0.5 (1)	+(1)	+	+(1)
Glutamic acid	4.3 (4)	3.2 (3)	1.0 (1)	6.5 (5)	9.0	5.0 (3)
Proline	0.9 (1)	1.3 (1)		5.6 (6)	6.1	6.8 (6)
Glycine	5.1 (5)	4.1 (4)		11.0 (10)	8.1	8.4 (8)
Alanine	3.4 (4)	2.9 (3)		11.0 (11)	3.2	9.6 (10)
Valine	3.9 (3)	3.1 (3)		9.2 (9)	7.2	10.6 (11)
Isoleucine		1.9 (2)		4.5 (4)	3.8	2.0 (2)
Leucine	2.7 (3)	2.4 (2)	1.0 (1)	10.0 (10)	5.6	10.6 (12)
Tyrosine	0.8 (1)	2.6 (3)	1.0 (1)	5.1 (5)	2.7	4.2 (4)
Phenylalanine	1.9 (2)	1.2 (1)		4.7 (4)	1.6	4.6 (5)
Tryptophan		+(2)		+(2)	+	+(2)
Carbohydrate				2.0[b]		1.1[b]
Total residues	34	45	4	117	(70)	118
Residue positions	1–34	35–79	80–83	84–204	(1–72)[c]	73–204
NH_2 terminus	PCA	His	Tyr	Asn	PCA	Ser

[a] Values are given in molar ratios. Figures in parentheses were deduced from sequence analysis; these are omitted for VDiB1 because the sequence is incomplete. [b] Values given are for glucosamine uncorrected for loss during acid hydrolysis. [c] The number of residues is approximate in some cases because the complete sequence of the CNBr fragment was not determined.

and the results obtained by carboxypeptidase assay (Table II) suggest the presence of three methionine residues in the variable region of the Ga μ chain, at least two in the Dau μ chain, but of only one in the variable part of the Di μ chain compared to four in the Ou μ chain. The probable disposition of these is shown in the alignment of the μ chains given in Figure 1.

Isolation of the Tryptic Peptides from CNBr Fragments. From the published sequence of the Ou μ chain (Putnam *et al.*, 1973) the largest CNBr fragment of the Fd region should be the one containing carbohydrate and ending with Met-204 (see later figure). If the C μ region begins at approximately Val-124, this fragment must have a minimum length of 80 residues from the C region plus the portion of the V region extending to the nearest arginyl residue. Fragments VGaB4 and VDiB2 met these criteria and were therefore digested with trypsin. The succinylated tryptic peptides of VGaB4 and VDiB2 were purified by gel filtration on a column of Sephadex G-75 in 1% NH_4OH (Figure 3). Because of succinylation of the lysyl residues, all these peptides should have COOH-terminal arginine except for the COOH-terminal peptide of each CNBr fragment which is recognizable by the homoserine in the carboxyl-terminal position. Four peaks containing tryptic peptides were identified in the elution pattern of the tryptic digest of CNBr fragments VGaB4 and VDiB2 (Figure 3). In each case the last peak contained salt plus two small tryptic peptides which had to be separated by paper electrophoresis. One of these was a heptapeptide

TABLE II: Carboxyl-Terminal Amino Acids of Tryptic Peptides and CNBr Fragments.[a]

	VGaB1T1	VGaB1	VGaB2	VGaB2T2	VGaB3	VDiB1[b]	VDiB1T1
Arginine	1.0			1.0		1.2	1.0
Threonine		0.9	1.1			1.4	
Serine		1.8				1.4	1.4
Homoserine		1.0	1.0		1.0	0.8	
Glutamic acid					0.9		
Glycine				0.9			
Alanine		1.2					
Isoleucine						1.0	1.2
Leucine					0.7		
Tyrosine		1.0			0.7		
Phenylalanine		1.6					
Tryptophan							1.2

[a] All peptides were incubated with a mixture of carboxypepticases A and B for 1 hr at 37°. [b] Carboxypeptidase B action stopped at lysine which was succinylated.

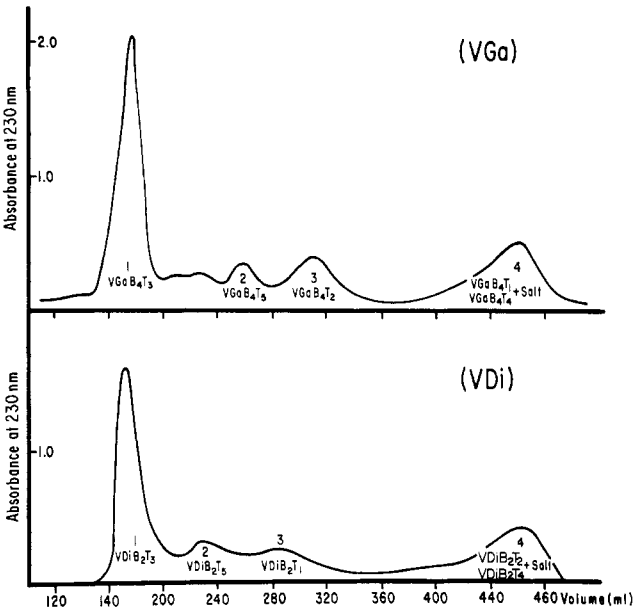

FIGURE 3: Separation of the tryptic peptides from the succinylated CNBr fragment including the switch area from the μ chain of IgM Ga (VGaB4, upper diagram) and IgM Di (VDiB2, lower diagram). Gel filtration was done on a column (2.5 × 100 cm) of Sephadex G-75 in 1% NH_4OH with a fraction size of 3.5 ml.

that was identical for both proteins (VGaB4T4 and VDiB2T4, respectively); this peptide corresponds to the segment of the C region from Gly-180 to Arg-186, *i.e.*, Gly-Phe-Pro-Ser-Val-Leu-Arg. The other peptide from the C region that was identical for both proteins was fraction 2 which contained VGaB4T5 and VDiB2T5, respectively; this is the COOH-terminal segment of the Fd' μ region and corresponds to the sequence from Gly-187 through Met-204. This set of peptides was identified by their amino acid composition and amino end groups but only VGaB4T5 was sequenced. The switch peptides (VGaB4T3 and VDiB2T3) were analyzed with the sequencer (see later). The amino acid composition, NH_2-terminal residues, and probable placement of the succinylated tryptic peptides of VGaB4 and VDiB2 are given in Tables III and IV, respectively.

The third peak eluted after gel filtration of the reduced-alkylated CNBr cleaved products of Fab Ga contained two fragments, VGaB1 and VGaB2 (Figure 2). The mixture was digested with trypsin, and the succinylated tryptic peptides were separated by gel filtration on Sephadex G-75 in 1% NH_4OH followed by further purification by paper electrophoresis at pH 1.9. Three peptides derived from VGaB1 were obtained (VGaB1T1, VGaB1T2, and VGaB1T3); the former had a blocked end group and therefore was assigned to the amino terminus of the chain (Table V). Four peptides later identified with VGaB2 were also purified (VGaB2T1, VGaB2T2, VGaB2T3, and VGaB2T4) (Table V). Since VGaB2T4 contained homoserine it must be carboxyl terminal.

Although some of the above peptides could be placed by homology to the Ou μ chain, they did not account for all of the amino acid residues in the fragments VGaB1 and VGaB2. It was therefore necessary to separate these fragments and purify the missing tryptic peptides. From Table I it is seen that VGaB2 lacks Cm-cysteine, which is present in VGaB1; this indicates the latter is linked elsewhere in the μ chain through a disulfide bond—presumably through an intrachain bridge to the second half-cystine in VGaB4 since VGaB1 is NH_2-terminal (see Figure 1). Accordingly, VGaB2 was prepared by CNBr cleavage of the unreduced Fab frag-

TABLE III: Amino Acid Composition of the Succinylated Tryptic Peptides Corresponding to the CNBr Fragment VGaB4.[a]

	VGaB4(T1 + T4)	VGaB4T2	VGaB4T3	VGaB4T5
Lysine			1.9 (2)	2.0 (2)
Histidine				
Arginine	2.0 (2)	0.8 (1)	1.1 (1)	
CM-cysteine		0.6 (1)	1.5 (2)	
Aspartic acid	1.0 (1)	1.4 (1)	9.1 (8)	1.1 (1)
Threonine		1.1 (1)	6.6 (7)	1.0 (1)
Serine[b]	2.1 (2)		12.8 (15)	1.9 (2)
Homoserine				+(1)
Glutamic acid		1.2 (1)	3.5 (3)	1.3 (1)
Proline	1.0 (1)		4.2 (4)	1.0 (1)
Glycine	2.0 (1)	0.9 (0)	6.7 (7)	2.1 (2)
Alanine		2.7 (3)	6.0 (6)	1.8 (2)
Valine	1.2 (1)	1.3 (1)	5.2 (5)	1.8 (2)
Isoleucine			3.6 (4)	
Leucine	2.2 (2)		5.6 (6)	1.6 (2)
Tyrosine		1.6 (2)	1.8 (2)	0.8 (1)
Phenylalanine	1.2 (1)		3.0 (3)	
Tryptophan			+(2)	
Carbohydrate			2.0[c]	
Total residues	11	11	77	18
Residue positions	(84–87) + (180–186)	88–98	99–179	187–204
NH_2 terminus	Asx + Gly	Ala	Ser	Gly

[a] Values are given in molar ratios. Figures in parentheses were deduced from sequence analysis. See also footnotes to Table I. [b] Serine values are uncorrected for loss in acid hydrolysis. [c] Values given are for glucosamine uncorrected for loss during acid hydrolysis.

TABLE IV: Amino Acid Composition of the Succinylated Tryptic Peptides Corresponding to the CNBr Fragment VDiB2.[a]

	VDiB2T1	VDiB2(T2 + T4)	VDiB2T3	VDiB2T5
Lysine			1.9 (2)	1.9 (2)
Histidine				
Arginine	0.9 (1)	2.0 (2)	1.5 (1)	
CM-cysteine	+(1)		1.5 (2)	
Aspartic acid	2.3 (3)		6.8 (7)	1.3 (1)
Threonine	4.0 (4)		6.7 (5)	1.2 (1)
Serine	3.3 (5)	0.6 (1)	12.2 (14)	2.2 (2)
Homoserine				+(1)
Glutamic acid			3.4 (2)	1.2 (1)
Proline		0.9 (1)	4.9 (4)	1.3 (1)
Glycine		0.9 (1)	4.3 (5)	1.8 (2)
Alanine	4.0 (4)		4.0 (4)	1.8 (2)
Valine	3.2 (3)	1.0 (1)	4.7 (5)	1.7 (2)
Isoleucine			1.5 (2)	
Leucine	2.8 (4)	1.2 (1)	4.1 (5)	1.7 (2)
Tyrosine	2.1 (2)		1.4 (1)	0.8 (1)
Phenylalanine	+(1)	1.0 (1)	2.5 (3)	
Tryptophan			+(2)	
Carbohydrate			1.7[b]	
Total residues	28	1 + 7	64	18
Residue position	73–100	115 + (180–186)	116–179	187–204
NH_2 terminus	Ser	Free Arg + Gly	Trp	Gly

[a] Values are given in molar ratios. Figures in parentheses were deduced from sequence analysis. [b] Values given are for glucosamine uncorrected for loss during acid hydrolysis.

ment of Ga. Gel filtration on Sephadex G-100 in 6 M urea yielded a major peak containing the light chain and VGaB1 and VGaB4 (all disulfide-bonded together) plus a second peak containing VGaB2. The first peak was reduced and alkylated and again passed through Sephadex G-100 in 6 M urea. In the second gel filtration the light chain and the large switch-region fragment VGaB4 were eluted together as the first peak, and VGaB1 separated as the second peak.

The purified fragment VGaB2 was digested with trypsin, and the tryptic peptides were purified by paper electrophoresis. Since VGaB2 was not succinylated, seven peptides were expected and found (three lysyl and three arginyl peptides plus the COOH-terminal fragment). This procedure enabled purification of all of the tryptic peptides in VGaB1 and VGaB2 except VGaB1T3; it also helped provide some of the overlaps of the tryptic peptides and aided assignment of the disulfide bridge. The amino acid composition, amino-terminal groups, and residue-position numbers of these peptides are given in Table V.

VDiB1, the succinylated CNBr fragment representing the NH_2-terminal end of the Di μ chain, could not be sequenced directly because of the blocked end group. Hence, VDiB1 was digested with trypsin, and the tryptic peptides were separated on Sephadex G-50 in 1% NH_4OH. Only two arginine residues were present and the corresponding peptides VDiB1T1, VDiB1T2, and VDiB1T3 were obtained (Figure 4). Amino acid analysis showed that the first peak on the column in Figure 4 represented the COOH-terminal portion of the Fd μ chain (residue positions 206–237). The amino acid analyses, amino-terminal groups, and residue positions of the succinylated peptides ascribed to VDiB1 are given in Table VII.

Because the blocked amino-terminal peptide VDiB1T1 was poorly characterized, an attempt was made to prepare the smaller unsuccinylated peptides. The Fab fragment was cleaved with CNBr, reduced, and alkylated, and the CNBr fragments were separated on Sephadex G-100 in 6 M urea. VDiB1 was digested with trypsin and a two-dimensional peptide map prepared at pH 1.9 by the method used previously in our laboratory for light chains (Putnam *et al.*, 1967). However, the peptides were reacted with trinitrobenzenesulfonic acid and the yellow peptide spots were eluted and analyzed. By this procedure, three additional peptides were isolated: VDiB1T1-1, VDiB1T1-2, and VDiB1T2-3 (Table VII). This still left gaps, so VDiB1 was digested with chymotrypsin. The chymotryptic peptides were likewise separated by the two-dimensional peptide map method, stained with trinitrobenzenesulfonic acid, eluted, and analyzed (Table VI).

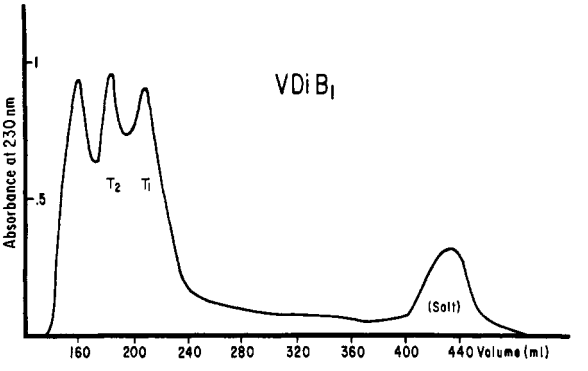

FIGURE 4: Separation of the tryptic peptides (T1 and T2) derived from VDiBl, the succinylated CNBr fragment that represents the N-terminal portion of the Di μ chain. Gel filtration was done on a column (2.5 × 100 cm) of Sephadex G-50 in 1% NH_4OH with a fraction size of 3.5 ml. The unlabeled first peak represents the C-terminal portion of the Fd piece of the Di μ chain (residues 206–237 in the Ou numbering system).

TABLE V: Amino Acid Composition of the Tryptic Peptides Corresponding to CNBr Fragments VGaB1 and VGaB2.[a]

	VGaB1T1	VGaB1T2	VGaB1T3	VGaB2T1	VGaB2T2-1	VGaB2T2-2	VGaB2T2-3	VGaB2T3	VGaB2T4	VGaB2T4-1	VGaB2T4-2
Lysine					1.1 (1)	0.9 (1)			0.9 (1)	1.1 (1)	
Histidine				0.8 (1)							
Arginine	1.0 (1)	1.0 (1)		1.0 (1)			1.0 (1)	1.1 (1)			
CM-cysteine			(1)								
Aspartic acid						2.1 (3)			3.2 (3)	1.9 (2)	1.1 (1)
Threonine			(1)					0.9 (1)	1.1 (1)		0.9 (1)
Serine	1.3 (1)	0.8 (1)	(4)			3.1 (3)		1.1 (1)	1.0 (1)	0.9 (1)	
Homoserine			(1)						0.6 (1)		0.6 (1)
Glutamic acid	4.1 (4)				0.9 (1)	2.2 (2)					
Proline	0.9 (1)				0.8 (1)						
Glycine	4.4 (4)		(1)		1.0 (1)	2.3 (2)	1.0 (1)				
Alanine	0.8 (1)		(3)		0.9 (1)	2.0 (2)					
Valine	3.5 (3)			1.1 (1)		1.7 (2)					
Isoleucine						0.9 (1)		1.0 (1)			
Leucine	1.3 (1)	1.1 (1)	(1)			2.0 (2)					
Tyrosine			(1)			2.4 (3)					
Phenylalanine			(2)					0.9 (1)			
Tryptophan				+(1)		+(1)					
Total residues	16	3	15	4	5	22	2	5	7	4	3
Residue positions	1–16	17–19	20–34	35–38	39–43	44–65	66–67	68–72	73–79	73–76	77–79
N terminus	blocked	Ser	Leu	His	Glx	Gly	Gly	Phe	Asx	Asx	Asx

[a] Values are given in molar ratios. Figures in parentheses were deduced from sequence analysis.

TABLE VI: Amino Acid Composition of the Chymotryptic Peptides from VDiB1.[a]

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14
Lysine				0.8 (1)				0.8 (1)				1.1 (1)		
Histidine														
Arginine								1.0 (1)				1.0 (1)	1.0 (1)	1.3 (1)
CM-cysteine														
Aspartic acid									1.0 (1)	1.2 (1)		0.8 (1)		
Threonine				1.0 (1)						1.8 (2)		1.0 (1)	0.8 (1)	1.1 (1)

Serine	0.9 (1)		1.0 (1)	1.0 (1)	1.0 (1)	1.0 (1)			1.0 (1)				1.8 (2)	1.0 (2)						
Homoserine													+(1)	+(1)		+(1)				
Glutamic acid	1.0 (1)		1.2 (1)		1.0 (1)			2.1 (2)	1.0 (1)				0.8 (1)							
Proline			1.0 (1)					1.5 (2)												
Glycine	2.0 (2)	1.8 (2)	1.0 (1)		1.5 (1)			1.8 (2)	1.0 (1)	1.2 (1)										
Alanine		1.1 (1)	0.8 (1)																	
Valine								0.8 (1)												
Isoleucine								0.6 (1)	1.0 (1)				0.9 (1)	0.8 (1)	1.0 (1)					
Leucine		1.2 (1)	1.2 (1)	2.2 (2)	1.3 (1)			1.0 (1)					1.0 (1)							
Tyrosine									0.6 (1)	0.7 (1)	1.0									
Phenylalanine																				
Tryptophan						+(1)	+(1)	+(1)												
Total residues	4	4	3	8	2	4	2	11	6	6	1	10	5	4						
Residue positions	6–9	8–11	9–11	13–20	28–29	35–38	37–38	39–49	50–55	56–61	62	63–72	68–72	69–72						
NH_2 terminus									Val	Ser										

[a] Values are given in molar ratios. Figures in parentheses were deduced from sequence analysis. See also footnotes to Table I.

Sequence Analysis of the V Region of the Ga μ Chain.[6] The strategy for sequence determination of the V regions of the μ chains consisted of three lines of attack: (1) isolation and analysis with the sequenator of all unblocked CNBr fragments having ten or more residues, (2) isolation of tryptic peptides of the CNBr fragments followed by either manual or automatic sequence analysis of only those peptides needed to extend the data on the CNBr fragments, (3) use of homology with published sequences of heavy chains to align CNBr fragments and tryptic peptides and to place residues in areas of undetermined sequence. Data for the sequencer results on the Fd region of the Ga μ chain are given in Table VIII, and the sequence data are summarized in Figure 5.

Partial Sequence of Cyanogen Bromide Fragment VGaB1. Because of the blocked end group, a direct determination could not be made of the sequence of the intact Ga μ chain, nor of the CNBr fragment VGaB1 or of its amino-terminal peptide VGaB1T1. The blocked residue was not removed from the μ chain by pyrrolidonecarboxylic acid peptidase,[7] but the enzyme was sufficiently active on VGaB1T1 to enable use of the sequenator for eight cycles on the pentadecapeptide prepared from it (Figure 5). The sequence of VGaB1T2 was established by the dansyl method and the specificity of trypsin (Figure 5). Sequenator analysis of VGaB1T3 gave data for ten steps (Table VIII). Methionine is placed at the carboxyl terminus of VGaB1 because of the liberation of homoserine by carboxypeptidase. The order of the tryptic peptides in VGaB1 is determined by the fact that VGaB1T3 contained carboxyl-terminal homoserine and VGaB1T1 was blocked.

Cyanogen Bromide Fragment VGaB2. Sequenator analysis corresponding to positions 35–73 was performed for 29 steps on VGaB2 with good yields and clear results on most of the steps (Table VIII and Figure 5). Digestion with carboxypeptidases A and B gave the carboxyl-terminal sequence Thr-Met for VGaB2 (Table II) and Ser-Arg at positions 71–72 (steps 37 and 38) for the tryptic peptide VGaB2T3. The sequencer analysis covered and aligned all of the tryptic peptides in VGaB2 and joined them to the Asx residue, which is the amino terminus of VGaB2T4. The partial sequence of VGaB2T4 was deduced from dansyl determinations on VGaB2T4-1 and VGaB2T4-2 (Table V) and the carboxypeptidase results on VGaB2 (Table II).

Cyanogen Bromide Fragment VGaB3. The sequence of this tetrapeptide was deduced by dansyl determination of the amino terminus as tyrosine and by carboxypeptidase digestion (Figure 5 and Table II).

Cyanogen Bromide Fragment VGaB4 and Tryptic Peptide VGaB4T3. Sequenator analysis of VGaB4 for 39 cycles gave clear results for the first 26 steps and for a number of steps thereafter up to the beginning of the C region (Table VIII and Figure 5). The last residue clearly identified was the isoleucine at position 120 (position 124 in the Ou sequence). In three other μ chains we had found this position to be valine and we had thought it marked the beginning to the C region. It was therefore essential to extend the sequence. This was done by sequenator analysis for 35 steps on the tryptic

[6] In the proof of sequence the residues in the V region of the Ga μ chain are numbered consecutively but in comparison to the Ou μ chain gaps are placed to maximize the alignment. All residues in the C region are given the Ou numberings. A more detailed proof of sequence has been submitted for examination to the reviewers and can be obtained by writing directly to the authors.

[7] Experiments with pyrrolidonecarboxylic acid peptidase were performed by Florence Dunne. The enzyme was supplied by Dr. Robert Fellows, Duke University School of Medicine, Durham, N. C.

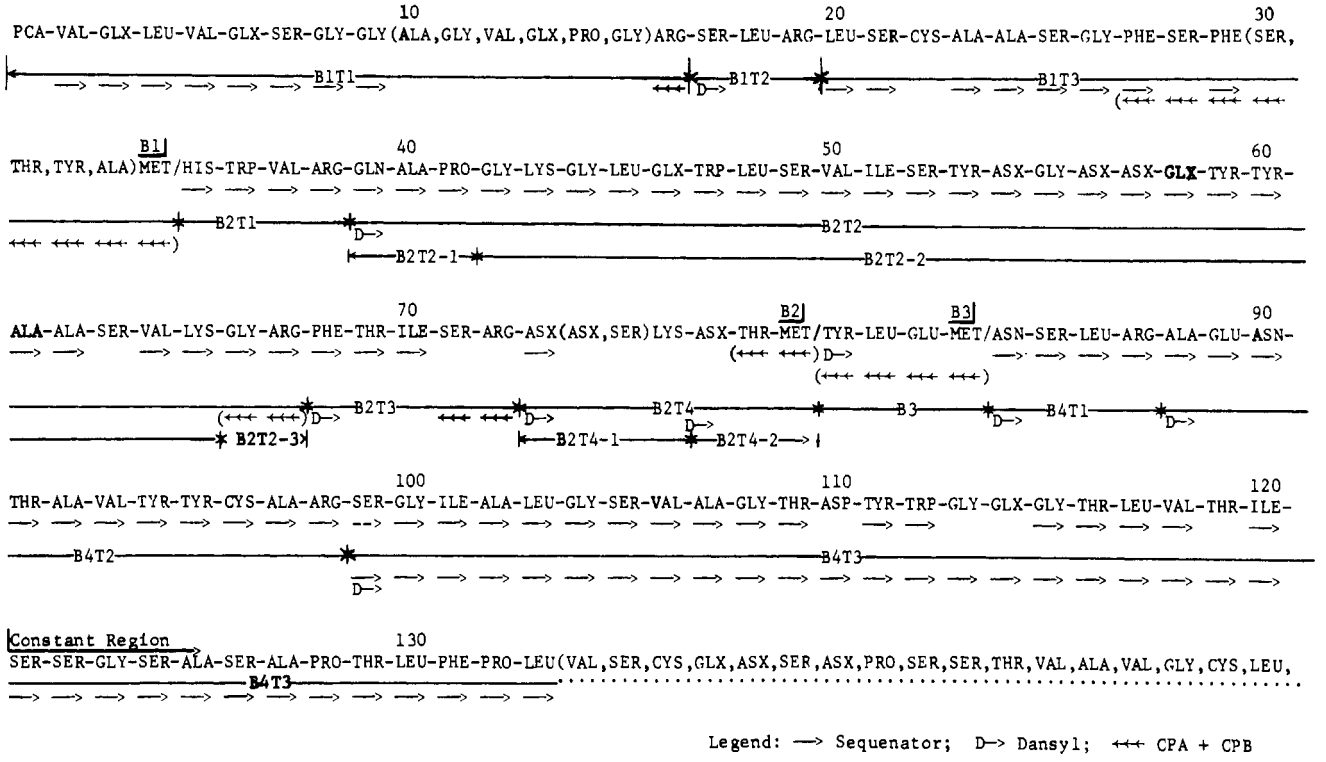

FIGURE 5: Amino acid sequence of the V region and of the beginning of the C region of the Ga μ chain.[6] Sequenator analysis of CNBr fragments VGaB2 (residues 35–79) and VGaB4 (residues 85–204) is shown by arrows (→) directly under the sequence for each residue determined. The tryptic peptides B1T1, B1T2, etc., are shown, and residues that were identified by various methods are indicated by symbols as follows: sequenator (→), dansyl method (D→), and digestion with carboxypeptidases A and B (←←←). The constant region begins on the bottom line with Ser-121 (Ser-125 in the Ou numbering system). Residues in parentheses on the bottom line represent the continuation of the constant sequence as determined in the Ou μ chain (Putnam *et al.*, 1973).

peptide VGaB4T3 (Table VIII). This gave an overlap with the parent CNBr fragment VGaB4 for 22 steps and extended the sequence into the C region for 13 steps after Ile-120, all of which are identical with the sequence established for the Ou μ chain.

Remaining Sequence of the Fd Region of the Ga μ Chain.

TABLE VII: Amino Acid Composition of Tryptic Peptides from VDiB1.[a]

	VDiB1T1	VDiB1T1-1	VDiB1T1-2	VDiB1T2	VDiB1T2-3	VDiB1T3
Lysine	1.4	0.9 (1)		2.3 (2)		
Histidine	1.1					
Arginine	1.0		0.8 (1)	0.8 (1)	1.2 (1)	
CM-cysteine	+					
Aspartic acid	1.1			3.1 (3)		
Threonine	2.0	0.8 (1)		2.0 (2)		1.0 (1)
Serine	4.9	1.2 (1)	0.7 (1)	3.1 (3)	1.0 (1)	
Homoserine						0.8 (1)
Glutamic acid	5.1	3.4 (3)	0.8 (1)	3.9 (4)		
Proline	1.0	1.4 (0)		3.3 (2)		
Glycine	6.1	2.0 (2)	1.1 (1)	4.8 (4)		
Alanine	2.3	1.1 (1)				
Valine	2.3	0.7 (1)	1.2 (1)	1.6 (1)		
Isoleucine	1.4		1.0 (1)	1.1 (1)		1.0 (1)
Leucine	4.2	2.5 (2)		2.4 (2)		
Tyrosine	1.4			1.9 (3)		
Phenylalanine	0.9					
Tryptophan	+		+(1)	+(1)		
Total residues	38	12	7	29	2	3
Residue number	1–40	1–12	34–40	41–67	68–69	70–72
N terminus	PCA	PCA		Gln		

[a] Values are given in molar ratios. Figures in parentheses were deduced from sequence analysis. Peptides VDiB1T1, VDiB2T2, and VDiB1T3 were from the succinylated fragment VDiB1; the others were from the unsuccinylated fragment.

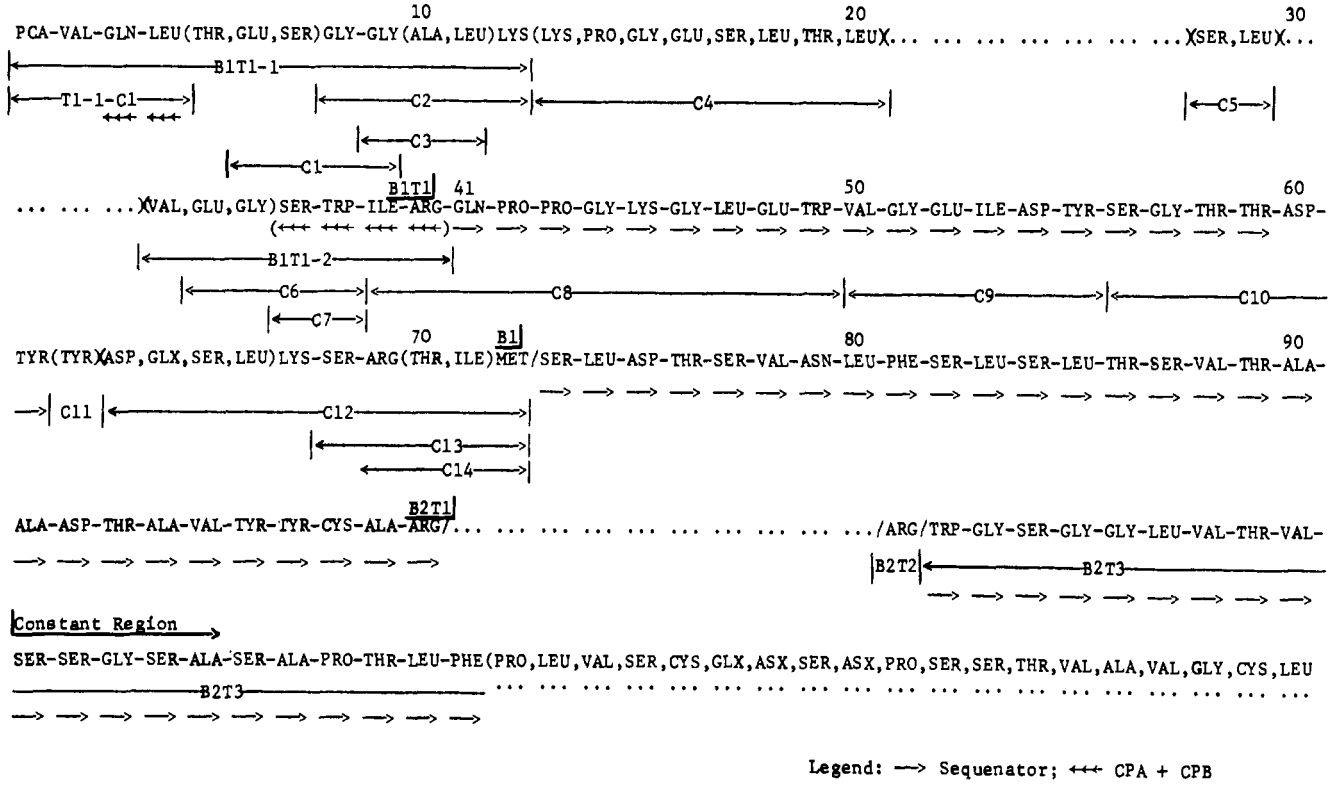

FIGURE 6: Amino acid sequence of the V region and of the beginning of the C region of the Di μ chain. Sequenator analysis of CNBr fragment VDiB1 and of tryptic peptide VDiB2T3 is shown by arrows (→) directly under the sequence for each residue determined. Other tryptic peptides (B1T1-1 etc.) and chymotryptic peptides (C1-C14) described in the text are identified. Dotted lines in parentheses indicate missing residues. The numbering system is based on homology to the V region of other heavy chains, the first invariant tryptophan being regarded as Trp-38. The constant region begins on the bottom line but the exact numbering is uncertain because of the gap after Arg-100. Residues in parentheses on the bottom line represent the continuation of the constant sequence as determined in the Ou μ chain (Putnam *et al.*, 1973).

The rest of the Fd sequence of the Ga μ chain was placed by homology to the Ou μ chain. VGaB4T4 with the sequence Gly-Phe-Pro-Ser-Val-Leu-Arg at positions 180–186 was present in the last peak of Figure 3. Sequenator analysis of VGaB4T5 gave very clear results for 12 steps and probable identification for three more as follows

Gly-Gly-Lys-Tyr-Ala-Ala-Thr-Ser-Glu-Val-Leu-Leu-Pro-Ser-Lys

This places the peptide in the sequence for residues 187–204 in the Ou μ chain (see Figure 9 later). Moreover, the residues (Ser,Glx) for which the order was uncertain in the Ou μ chain were placed in sequence as Ser-Glu, each residue being identified both by gas chromatography and by amino acid analysis after regeneration.

Alignment of CNBr Fragments of the Ga μ Chain. VGaB1 is placed at the N-terminus of the Ga μ chain because both have blocked end groups and because VGaB1 has the characteristic invariant half-cystine at position 22 and a methionine residue at position 34. VGaB2 is placed as the second fragment in the Ga μ chain because of homology and such characteristic features as the invariant tryptophans at positions 36 and 47. VGaB3 provides a characteristic methionine residue for position 83. The sequence after this is continuous from the N-terminus of CNBr fragment VGaB4 into the C region. VGaB1 and VGaB4 are linked through an intrachain disulfide bridge. Slant lines are used in Figure 5 to indicate that the order of VGaB2 and VGaB3 was not established.

Sequence Analysis of the V Region of the Di μ Chain. Amino Terminus. The blocked amino terminus indicates that VDiB1 is the NH_2-terminal CNBr fragment of the Di μ chain (Figure 6). The blocked amino-terminal tetrapeptide (T1-1C1) was reported previously by Köhler *et al.* (1970). The succinylated tryptic peptide (VDiB1T1) could not be sequenced because of the blocked end group but digestion of VDiB1T1 with carboxypeptidases A and B (Table II), together with the data on C-7 (Table VII), gave the COOH-terminal sequence of Ser-Trp-Ile-Arg. The chymotryptic peptides C-1–C-7 (Table VII) were tentatively placed by homology to the Ou and Ga μ chains (see later figure).

Residues 41–72 in CNBr Fragment VDiB1. As shown in Figure 6, this portion of VDiB1 was established by sequencer analysis of VDiB1T2 (Table VIII) supplemented by amino acid analysis of the chymotryptic peptides C-8–C-14 (Table VII) and carboxypeptidase digestion of VDiB1 (Table II). C-8 gave the overlap to VDiB1T1. The COOH-terminus of VDiB1 is given by the overlapping peptides C-12–C-14 (Table VII) and by digestion of VDiB1 with carboxypeptidase. VDiB1T3 must be carboxy-terminal in VDiB1 because of the presence of homoserine.

Residues 73–100. Tryptic Peptide VDiB2T1. The complete sequence of VDiB2T1 was determined with the sequenator (Table VIII, Figure 6). No overlap of the CNBr fragments VDiB1 and VDiB2 was obtained. However, VDiB2T1 must be amino-terminal in VDiB2 because of the serine end group (Tables I and IV) and the presence of the characteristic sequence of most heavy chains for positions homologous to 91–99, *i.e.*, Asp-Thr-Ala(X)Tyr-Tyr-Cys-Ala-Arg (Putnam *et al.*, 1972).

Hypervariable Deletion Region. Despite a careful search, no peptide was found in the tryptic digest of VDiB2 that corresponded to the section designated positions 100–114 in the Ou μ chain (*i.e.*, immediately after Cys-Ala-Arg). In other

TABLE VIII: Automatic Edman Degradation of Peptides.[a]

	VGaB1T1			VGaB1T3			VGaB2			
Position:	1–16			20–34			35–79			
Analysis:	2–9			20–29			35–73			
Step No.	Amino Acid	Glc	Yield (%)	Step No.	Amino Acid	Glc	Yield (%)	Step No.	Amino Acid	Glc	Yield (%)
1		Val	100	1		Leu	100	1		His	
2	Glx			2		Ser		2		Trp	60
3		Leu	90	3		(CmC)[b]		3	Val	Val	61
4		Val	100	4		Ala	52	4	Arg		
5	Glx			5		Ala	55	5		Glx	
6		Ser		6		Ser		6		Ala	100
7		Gly	45	7		Gly	10	7		Pro	27
8		Gly	19	8		Phe	12	8	Gly	Gly	30
				9		(Ser)[c]		9	Lys		
				10		Phe	6	10		Gly	25
								11		Leu	50
								12		Glx	
								13		Trp	10
								14		Leu	45
								15		Ser	
								16		Val	55
								17		Ile	45
								18		Ser	
								19		Tyr	73
								20	Asx		
								21		Gly	14
								22	Asx		
								23	Asx		
								24	Glx		
								25		Tyr	73
								26		Tyr	41
								27		Ala	20
								28	Ala	Ala	20
								29	(Ser)		
								30		Val	18
								31	Lys		
								32		Gly	
								33	Arg		
								34		Phe	11
								35		Thr	
								36		Ile	11
								37	(Ser)[d]		
								38	(Arg)[d]		
								39	Asp		

heavy chains this section is denoted the hypervariable deletion region (Putnam *et al.*, 1971, 1972) because it is characterized by numerous deletions and a remarkable diversity in amino acid sequence. Because overlaps between VDiB2T1, VDiB2T2, and VDiB2T3 were not established, the sequences found for VDiB2T3 are not numbered.

Switch Region. Peptide VDiB2T3. The presence of the switch region in VDiB2T3 was verified by sequenator analysis for 20 steps to the phenylalanine residue, which is 11 residues into the C region (Table VIII, Figure 6). The high serine content at the beginning of the C region (eight serines in the first 23 residues) thwarts a more extended analysis.

COOH-terminal Portion of Fd'. Residues 180–204. VDiB2T4 and VDiB2T5 (Table IV) complete the Fd region of the Di μ chain. VDiB2T4 is the same as VGaB4T4 and represents the sequence Gly-Phe-Pro-Ser-Val-Leu-Arg for positions 180–186 in the Ou μ chain. VDiB2T5 and VGaB4T5 are identical and represent the sequence established for positions 187–204 at the COOH-terminus of the Fd fragment in the Ou μ chain.

Glycopeptide Containing Oligosaccharide C1. A glycopeptide with the sequence Phe-Ser-Trp-Lys-Tyr(Asx, Asx, Ser, Asx, Lys) and containing the complex oligosaccharide C1 was isolated from the Di μ chain (Shimizu *et al.*, 1971). An identical glycopeptide is present in the Ou μ chain at positions 165–174 (see later figure) and probably in IgM Dau (Hurst *et al.*, 1973).

Discussion

The sequence data on μ chains presented herein, together with complete sequences of other workers on γ chains and

TABLE VIII (*Continued*)

Position:	VGaB4 84–204			VGaB4T3 99–179			VDiB1T2 41–72		
Analysis:		84–122			99–133			41–59	
Step No.	Amino Acid	Glc	Yield (%)	Step No.	Amino Acid	Glc	Yield (%)	Step No.	Amino Acid	Glc	Yield (%)
1		Asn		1		Ser		1		Gln	
2		Ser		2		Gly	100	2		Pro	58
3		Leu	100	3		Ile	64	3		Pro	58
4	Arg			4		Ala	64	4		Gly	100
5		Ala	88	5		Leu	49	5	Lys		
6		Glu		6		Gly	85	6		Gly	58
7		Asn		7		Ser		7		Leu	100
8		Thr	28	8		Val	42	8	Glx	Glu	
9		Ala	42	9		Ala	53	9		Trp	14
10		Val	87	10		Gly	55	10		Val	61
11		Tyr	76	11		Thr		11		Gly	30
12		Tyr	70	12	Asx	Asp		12	Glx		
13		CmC		13		Tyr		13		Ile	61
14		Ala	51	14		Trp	24	14	Asx		
15	Arg			15	Gly	Gly	42	15		Tyr	52
16		(Ser)[e]		16	Glx	Glx		16		Ser	
17		Gly	25	17		Gly	9	17		Gly	14
18		Ile	70	18		Thr		18		Thr	
19		Ala	18	19		Leu	16	19		Thr	
20		Leu	48	20		Val	11				
21		Gly	12	21		Thr					
22		Ser		22	Ile	Ile	11				
23		Val	12	23		Ser					
24		Ala	13	24		Ser					
25		Gly	14	25		Gly	7				
26		Thr	4	26		Ser					
27		(Asp)[e]		27		Ala	6				
28		Tyr	22	28		Ser					
29		Trp		29		Ala	5				
30		(Gly)[e]		30		Pro	2				
31		(Glx)[e]		31		Thr					
32		Gly	2	32		Leu	2				
33		Thr	3	33		Phe	2				
34		Leu	13	34		Pro	1.3				
35		Val	9	35		Leu	1.7				
36		(Thr)[e]									
37	Ile	Ile	9								
38		(Ser)[e]									
39		Ser									

partial sequence data of our laboratory and others on α chains, afford a comprehensive picture of the structural characteristics of human heavy chains, which is probably valid for heavy chains of all species. The V region of heavy chains regardless of class (V_H) may be pictured schematically (Figure 7) as being composed of three alternating segments that are characteristic of the V_H subgroup and independent of the class of the C region. The subgroup-characteristic segments are intercalated among three hypervariable regions that are related to antibody specificity. Of the latter, the largest and most important is the hypervariable deletion region (positions 100–115) which probably determines much of the antigen-combining site of the heavy chain (Ray and Cebra, 1972). The hypervariable deletion region is preceded by a conservative segment (positions 91–99) and is followed by the switch point joining the V and C regions. Paradoxically, the subgroup-characteristic and conservative segments of the V region have been more stable in evolution than have the C regions that define the classes of heavy chains. These characteristics of heavy chains, as revealed by μ chain sequence data, will be discussed beginning with the evidence that the V regions of μ and γ chains are comparable in length but are not terminated or followed by a common sequence that might serve as a recognition for union of V_H genes with C genes.

Although much evidence for V and C regions in human μ chains has been adduced from peptide maps (Putnam *et al.*, 1967) and from partial sequence data obtained on unblocked μ chains[8] by use of the sequenator (Köhler *et al.*, 1970; Wang

[8] Although most heavy chains of the V_{HIII} subgroup are not blocked at the amino terminus, two chains (Nie and Ga) for which the V region sequence has been completed or nearly completed are blocked.

TABLE VIII (*Continued*)

Position:		VDiB2T1 73–100					
Analysis:		73–100		VDiB2T3			
Step No.	Amino Acid	Glc	Yield (%)	Step No.	Amino Acid	Glc	Yield (%)
1		Ser		1		Trp	100
2		Leu	52	2		Gly	66
3		Asp		3		Ser	
4		Thr		4		Gly	54
5		Ser		5		Gly	27
6		Val	100	6		Leu	83
7		Asx		7		Val	57
8		Leu	50	8		Thr	
9		Phe	51	9		Val	48
10		Ser		10		Ser	
11		Leu	76	11		Ser	
12		Ser		12		Gly	20
13		Leu	48	13		Ser	
14		Thr		14		Ala	24
15		Ser		15		Ser	
16		Val	80	16		Ala	17
17		Thr		17		Pro	12
18		Ala	42	18		Thr	
19		Ala	40	19		Leu	10
20		Asp		20		Phe	11
21		Thr					
22		Ala	28				
23		Val	46				
24		Tyr	15				
25		Tyr	30				
26		CmC					
27		Ala	36				
28	Arg						

[a] The yields are based on gas–liquid chromatography data. Because absolute molecular weights of peptides were not determined, yields are based on the first amino acid present with the largest amount. The yields are determined only for the amino acids present in the standard. Residues that were uncertain in the sequenator analysis and identified by other procedures are placed in parentheses. [b] Not identified with sequencer; placed by homology to other heavy chains from amino acid composition of tryptic peptide. [c] Determined by carboxypeptidase (Table II). [d] Determined by carboxypeptidase on VGaB2T3. [e] For placement see sequencer analysis of VGaB4T3.

et al., 1970, 1971; Capra, 1971), the length of the V region and the location of the switch point from the V to the C regions in μ chains were not previously established. Partly on the basis of unpublished data and partly from homology to the γ heavy chain, Putnam *et al.* (1971, 1972) had proposed that the C region of human μ chains begins with Val-124 in the Ou μ chain sequence. The present studies on three additional human μ chains suggest that the C region begins one residue later at Ser-125 in the Ou numbering system. Thus, in the switch region, *i.e.*, the juncture of the V and C regions, human μ and γ chains share at most only the Ser-Ser sequence. This is illustrated in Figure 8 showing the sequence at the switch region for the four μ chains studied by us in comparison with the sequence of five human γ1 chains and one γ3 chain studied by other workers. This short dipeptide sequence corresponding to only six nucleotide bases seems an insufficient recognition signal for the union of V and C genes.

Of course, as more human μ chains are sequenced completely, the length of the V region may be extended by one or two more residues, as was the case for human κ light chains (Putnam, 1969). Indeed, Figure 8 suggests that Val-115, which begins the C region of human γ chains (in the Eu numbering system), may be substituted in other γ chains, just as the corresponding position of Val-124 is in the Ga μ chain.

Because of the presence of the hypervariable deletion region at residues 100–115, the length of the V region of human heavy chains is somewhat longer (about 114–124 residues) than the V region of human κ and λ light chains, which is about 108 residues. For example, most human λ light chains have a V region of 108 residues but in some cases the λ V region may be as short as 105 residues and in others as long as 112 residues (Putnam, 1970). In human γ heavy chains thus far sequenced the V_H region varies in length from 114 to 118 residues (Edelman *et al.*, 1969; Cunningham *et al.*, 1969; Press and Hogg, 1970; Ponstingl and Hilschmann, 1972). The Ou μ chain has the longest V_H region so far recorded (124 residues), and the V_H region of the Ga μ chain is 120 residues; however, the V_H region of the Di μ chain may be as short as 108 residues if the apparent deletion from 100 to 114 is real. In heavy chains of normal unimmunized animals the hypervariable deletion region has not been determined because of the multiple sequences (Fruchter *et al.*, 1970; Bourgois and

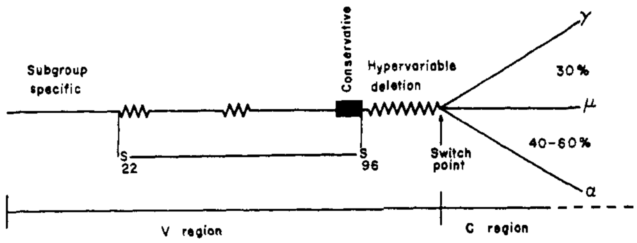

FIGURE 7: Schematic diagram of the structural features common to μ, γ, and α heavy chains. The V region (114–124 residues) consists of a subgroup-specific region (90 residues) followed by a conservative region (positions 91–99) and a hypervariable deletion region. The subgroup-specific region consists of three segments intercalated among three hypervariable regions. The latter are indicated by zigzag lines after Cys-22, around position 60, and after position 99. Because of variation in length of the hypervariable deletion region, and also because of subgroup-specific gaps in the first 90 residues, the V region varies in length. However, at the switch point a constant sequence characteristic of the class of the chain is initiated. The C region of the human γ1 chain has 332 residues, and the C region of the α1 chain probably has a similar length. However, the C region of the human μ chain has 452 residues (Putnam *et al.*, 1973). The human μ and γ chains have about 30% identity in sequence, whereas the sequence identity of the human μ and α chains is higher, especially at the COOH terminus. Chains with different C regions but belonging to the same V region subgroup may have twice as much homology in the V region as in the C region.

the composition and properties of the peptides obtained for the remainder of the Fd′ region through Met-204 exclude any major differences in structure (Figure 9). Furthermore, the sequence around the light–heavy interchain disulfide bridge is identical in a number of other human μ chains (Frangione *et al.*, 1971; Metzger, 1970). Of course, small allotypic differences in sequence of the Fd′ portion of the C region of the μ chain are not excluded.

Discussion regarding the homology of the C region of μ, γ, and α chains will be reserved for a later paper describing more extensive studies on the sequence of the Fc region of a series of human μ chains.[9] Some figures illustrating the homology of μ and γ chains have already been published (Putnam *et al.*, 1971, 1972), and data regarding the homology of the COOH-terminal portion of μ, γ, α, and ε heavy chains are in press (Bennich *et al.*, 1973; Putnam *et al.*, 1974). The results are shown schematically for μ, γ, and α chains in Figure 7. Whereas μ and γ chains have about 30% identity in sequence (excluding a few gaps and omitting one of the five homology regions of the μ chain), μ and α chains have 55–60% homology in the last 50 residues of their C regions (Chuang *et al.*, 1973; Putnam *et al.*, 1973). However, preliminary data of our laboratory indicate that this high degree of homology does not extend throughout the C regions of the μ and α chains (Put

```
                                115                 120            124
                                                               VARIABLE    CONSTANT
Ou     μ   VHII     Met-Asp-Val-Trp-Gly-Lys-Gly-Thr-Thr-Val-Thr-Val-Ser-Ser

Ga     μ   VHIII    ——  Asp-Tyr-Trp-Gly-Gln-Gly-Thr-Leu-Val-Thr-Ile-Ser-Ser
                                                                              Gly-Ser-Ala-Ser-Ala
Di     μ   VHI      ——  ——  Arg-Trp-Gly-Ser-Gly-Gly-Leu-Val-Thr-Val-Ser-Ser

Dau    μ   ?        ——  Asp-Val-Trp-Thr-Ser-Gly-Thr-Thr-Val-Thr-Val-Ser-Ser


                                106             110                 115
Eu     γ1 VHI       ——  ——  Glu-Glu-Tyr-Asn-Gly-Gly-Leu-Val-Thr-Val-Ser-Ser

Cor    γ1 VHII      Met-Asp-Val-Trp-Gly-Arg-Gly-Thr-Pro-Val-Thr-Val-Ser-Ser

Daw    γ1 VHII      Phe-Asp-Tyr-Trp-Gly-Gln-Gly-Ile-Leu-Val-Thr-Val-Ser-Ser
                                                                              Ala-Ser-Thr-Lys-Gly
He     γ1 VHII      Phe-Asp-Val-Trp-Gly-Gln-Gly-Thr-Lys-Val-Ala-Val-Ser-Ser

Nie    γ1 VHIII     Phe-Ala-His-Trp-Gly-Gln-Gly-Thr-Leu-Val-Thr-Val-Ser-Ser

Jon    γ3 VHIII     Met-Asp-Val-Trp-Gly-Gln-Gly-Thr-Pro-Val-Thr-Val-Ser-Ser
```

FIGURE 8: The switch region of four human μ chains reported in this work compared to human γ1 and γ3 chains reported by other workers (Eu, Edelman *et al.*, 1969; Cor and Daw, Press and Hogg, 1970; He, Cunningham *et al.*, 1969; Nie, Ponstingl and Hilschmann, 1972). The numbering system given is for the Ou μ chain and for the Eu γ chain. The V region varies in length for heavy chains, *e.g.*, in the Eu γ1 chain, the C region begins with Val-115.

Fougereau, 1970). This is also a site of extensive deletions in defective heavy chains from patients with "heavy-chain disease" (Franklin and Frangione, 1971).

All four μ chains studied differ in sequence and probably all differ in length of the V region, but in the C region beginning with Ser-125, the three studied have identical sequence through Phe-135 and in all of the many additional segments thereafter (including much of the Fc region) for which we have thus far completed the structure.[9] Although the sequence of the Ga, Di, and Dau μ chains is incomplete in the area around and after the light-heavy disulfide bridge at Cys-140,

nam *et al.*, 1974). In contrast, the V regions of heavy chains, regardless of class, may have up to 70% homology if they are of the same subgroup. In fact, the V region of our μ chain Ga differs from the V region of the γ1 chain Nie (Ponstingl and Hilschmann, 1972) by only 13 out of the first 98 residues if the two are aligned for maximum homology without any gaps.

The remaining discussion will consider the structural characteristics of the V region of μ chains beginning with the hypervariable deletion region. This region has been implicated as the major determinant of the antibody combining site of heavy chains by a number of workers using various techniques (Porter, 1973; Benjamin *et al.*, 1972). As a result, much discussion has ensued, including our own comparisons of the sequence of this region in a number of human and

[9] Florent, G., Lehman, D., Lockhart, D., and Putnam, F. W., submitted to *Biochemistry*.

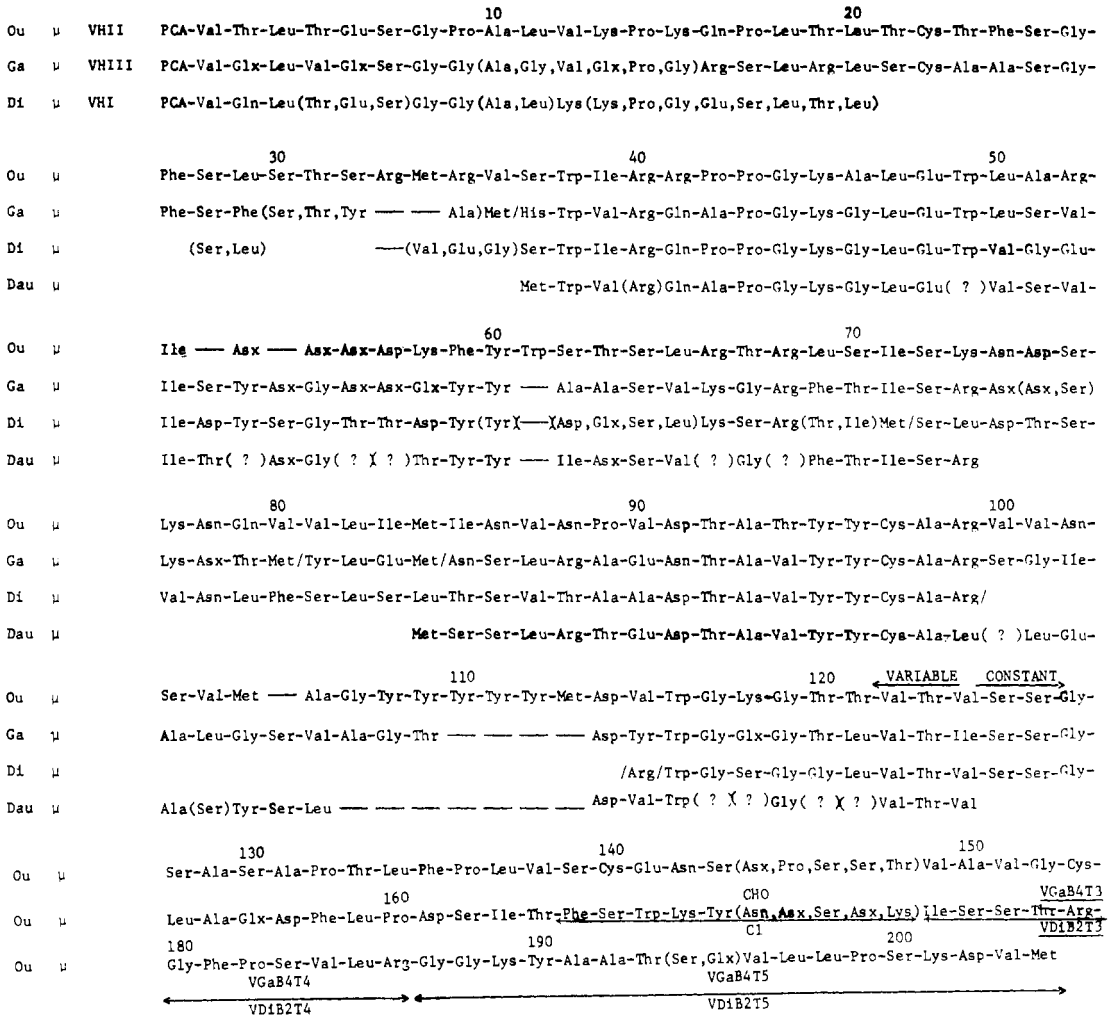

FIGURE 9: Comparison of the sequences of the V region of four human μ chains (Ou, Ga, Di, and Dau). The sequence is also given for the Ou μ chain through Met-204, and the Ou numbering system is used (Putnam *et al.*, 1973). The position of various peptides from the C region of the Ga and Di chains is also shown. The question marks in the Dau sequence indicate serine and threonine residues that were tentatively identified by gas chromatography but which need further verification. The probable sequence of the Dau μ chain from positions 114 to 124 (in the Ou numbering system) is Asp-Val-Trp-Thr-Ser-Gly-Thr-Thr-Val-Thr-Val-Ser-Ser. Position 100 is probably arginine.

animal heavy chains (Putnam *et al.*, 1970, 1971, 1972). The additional results on μ chains shown in Figures 8 and 9 validate the designation of the segment from position 100–115 as a region of hypervariability and of deletions. Although the Ou and Ga μ chains are identical at half of their first 100 positions, a series of arbitrary gaps has to be inserted to align a single pair of residues in the next 15 positions. Also, the Ga μ chain is three residues shorter than the Ou μ chain in the area from the invariant Ala-98 through the almost invariant Trp-116. Similar considerations apply to the Dau μ chain, and, as was noted earlier, no peptides for this area were recovered for the Di μ chain.

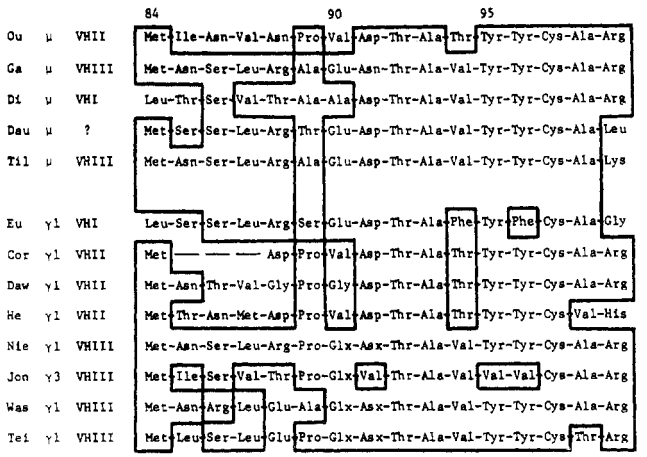

FIGURE 10: Comparison of the sequences of five human μ chains and eight human γ chains in the segment from Met-84 through Arg-99 in the Ou numbering system. Although the first few positions are quite variable, the sequence Asx-Thr-Ala-Val-Tyr-Tyr-Cys-Ala-Arg is highly conserved for all three subgroups. Sources of data for Eu, Cor, Daw, He, and Nie as in Figure 8. Data for Jon, Tei, and Was from Kehoe and Capra (1971) and for Til from Wang *et al.* (1973).

In contrast to the hypervariable deletion region is the conservative region just preceding it from positions 90 to 99. Here the prototype sequence is Asx-Thr-Ala-Val-Tyr-Tyr-Cys-Ala-Arg; there are few deviations whether the chain is μ or γ, whether V_{HI}, V_{HII}, or V_{HIII} (Figure 10) or even animal or human (Putnam *et al.*, 1972). Kehoe and Capra (1971) examined all V_H sequences available at that time and concluded that *no* subgroup-specific sequences could be identified from position 96 to the beginning of the constant region of a series of heavy chains of different classes. They suggested that the last subgroup-specific residue of V_H appears to be 94, *i.e.*, phenylalanine for V_{HI}, threonine for V_{HII}, and valine for V_{HIII}. However, our Di μ chain has valine at position 94; yet though difficult to type, this chain resembles the V_{HII} subgroup in sequence more closely than it does V_{HIII}. From the

TABLE IX: Matrix Table of Identities in the First 100 Residues of the Subgroup Specific (V_H) Region of Human μ and γ Chains.[a]

Subgroup	Class	Protein	Identities						
V_{HII}	μ	Ou	100						
V_{HII}	$\gamma 1$	Cor	75	100					
V_{HII}	$\gamma 1$	Daw	72	79	100				
V_{HII}	$\gamma 1$	He	61	65	64	100			
V_{HIII}	$\gamma 1$	Nie	47	47	45	40	100		
V_{HIII}	μ	Ga	48	49	49	42	81	100	
V_{HI}	$\gamma 1$	Eu	31	34	30	28	53	50	100
Protein			Ou	Cor	Daw	He	Nie	Ga	Eu
Class			μ	$\gamma 1$	$\gamma 1$	$\gamma 1$	$\gamma 1$	μ	$\gamma 1$
Subgroup			V_{HII}	V_{HII}	V_{HII}	V_{HII}	V_{HIII}	V_{HIII}	V_{HI}

[a] To simplify comparisons some assumptions have been made with respect to amide groups, and some residues in the Ga μ chain have been placed by homology.

tabulation of sequences given in Figure 10 we conclude that the subgroup classification does not extend beyond residue 90.

The three heavy chain subgroups in human immunoglobulins (V_{HI}, V_{HII}, and V_{HIII}) have previously been defined largely by reference to the complete V_H sequences of the Ou μ chain and of five $\gamma 1$ chains[10] and by amino-terminal sequences of from 20 to 40 residues obtained by sequenator analysis of many μ, γ, and α chains of the unblocked V_{HIII} subgroup (Köhler *et al.*, 1970; Putnam *et al.*, 1971, 1972; Wang *et al.*, 1970, 1971; Capra, 1971). The Ga μ chain clearly belongs to the V_{HIII} subgroup as is shown by Table IX which gives a matrix comparison of the number of identities in the first 100 residues of the subgroup-specific (V_H) region of two human μ and five $\gamma 1$ heavy chains.[10] Whereas the first 100 residues of the Ga μ chain have at least 80% identity in sequence with the Nie $\gamma 1$ chain of subgroup V_{HIII}, there is somewhat less than 50% identity with the corresponding portion of the Ou μ chain and with $\gamma 1$ chains of the V_{HII} subgroup and about 50% identity with the similar portion of the Eu $\gamma 1$ chain of the V_{HI} subgroup. This reaffirms the conclusion earlier based on complete sequence data for only one μ chain (Ou) that V region sequences of heavy chains can be placed into subgroups independent of the class of the C region. From Table IX it is evident that V_{HIII} subgroup sequences (Ga and Nie) are intermediate between V_{HI} and V_{HII} and are almost equally related to the latter two subgroups. These interrelationships partly reflect the fact that 21 of the first 100 residues are identical in the two μ chains and the five $\gamma 1$ chains, that is, about one-fifth of the V region sequence is conserved regardless of the subgroup.

Although the partial sequence data given on the Dau μ chain in Figure 9 indicate that Dau like Ga is in the V_{HIII} subgroup, it is much more difficult to make a subgroup assignment from the partial sequence data for the Di μ chain. In the segment from Trp-38 through Arg-99 the Di μ chain has almost equal homology with the subgroup V_{HI} chain Eu (37%), with the subgroup V_{HIII} chains Ga and Nie (45 and 41%, respectively), and with the subgroup V_{HII} chain He (37%). However, in the same segment the Di μ chain has 53% homology with the more representative subgroup V_{HII} chain Cor including identity of the decapeptide sequence from Trp-38 through Trp-49. Hence, though we at first thought the Di μ chain was of the V_{HI} subgroup because of the partial sequence close to the amino terminus (Köhler *et al.*, 1970), from more extensive data it appears more related to V_{HII}. This inability to assign a subgroup unambiguously although half the sequence of the V region has been determined casts some doubt on the sharpness of the definition of subgroups. It also raises question about the validity of the assignment of many heavy chains to subgroups on the basis of amino-terminal sequences of only 20–30 residues.

The identification of the subgroup of a heavy chain would be much easier if certain residues at a number of positions were unfailingly characteristic of the subgroup. Despite a careful review of published sequence data and the new results on μ chains herein, we are unable to identify a set of such subgroup-specific residues. The following residues are highly characteristic of subgroup V_{HIII} in that they are common to Nie and Ga but absent in Ou, Cor, Daw, He, and Eu: Gly-9, Gln-13, Arg-16, Arg-19, Ala-23, and Tyr-32. However, when these positions are checked against the 20 V_{HIII} heavy chains listed by Wang *et al.* (1970, 1971) and Capra (1971) for which data are available for positions 1–35, only Gly-9 was unique for V_{HIII}. All the other positions varied in one or more of the V_{HIII} chains. A similar situation would probably prevail if more extensive data were available after position 35. Thus, as additional data accumulate, it is getting more difficult to identify any positions that are absolutely characteristic for the subgroup. Though subgroups represent a convenient classification for the bewildering variety of heavy-chain V region sequences, intermediate sequences occur with some restrictions at some positions. Increasingly, subgroups appear to reflect overlapping multimodal distributions rather than a small number of segregated sets of sequences. This conclusion has considerable implications for current theories of the origin of immunoglobulin genes—whether by evolution, somatic mutation, or somatic mutation and recombination. It is more in accord with the "germline" theory than with recombination theory which excludes crossing-over between genes specific for each subgroup.

Although the lack of subgroup-specific residues suggests extensive evolutionary divergence or extraordinary somatic mutation of heavy chain V genes, the V_H genes also have strong conservative features. In fact, V_H genes seem more conservative than C_H genes on an evolutionary basis, for the two μ and five $\gamma 1$ V_H regions completely sequenced have 21 residues in common, which is much more than the three Cγ and four Cμ homology regions do. Despite many similarities in primary structure, all 14 of these homology regions (7 V_H, 3

[10] For a listing of these sequences see Figure 5 of Putnam *et al.* (1972) or Table III of Wang *et al.* (1973).

Cγ, and 4 Cμ) have only three common residues: the two half-cystines in the intrachain disulfide bridge and the tryptophan residue some 14 positions after the first half-cystine.

The new sequence data for three additional μ chains summarized herein (Ga, Di, and Dau) extend to μ chains the evidence for the switch region earlier established for γ chains. The results show that the variable (V_H) regions of μ and γ chains are of comparable length and may have very close homology in amino acid sequence—much closer in the case of members of the same subgroup than exists between the C regions of different classes. This finding supports the concept that there is a switch in biosynthesis of IgM antibody in the primary response to IgG antibody in the secondary response, that need only involve a shift from synthesis of the C region of the μ chain to the C region of the γ chain (Wang *et al.*, 1973). Such a switch is explained by the hypothesis of separate genes for the V and C regions of heavy chains.

References

Benjamin, D. C., Hussain, Q. Z., and Cebra, J. J. (1972), *Biochemistry 11*, 3641.

Bennich, H., Milstein, C., and Secher, D. S. (1973), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 33*, 49.

Bourgois, A., and Fougereau, M. (1970), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 8*, 265.

Capra, J. D. (1971), *Nature (London), New Biol. 230*, 61.

Chuang, C. Y., Capra, J. D., and Kehoe, J. M. (1973), *Nature (London) 244*, 158.

Cunningham, B. A., Pflumm, M. N., Rutishauser, U., and Edelman, G. M. (1969), *Proc. Nat. Acad. Sci. U. S. 64*, 997.

Edelman, G. M., Cunningham, B. A., Gall, W. E., Gottlieb, P. D., Rutishauser, U., and Waxdal, M. J. (1969), *Proc. Nat. Acad. Sci. U. S. 63*, 78.

Frangione, B., Prelli, F., Mihaesco, C., and Franklin, E. C. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 1547.

Franklin, E. C., and Frangione, B. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 187.

Fruchter, R. G., Jackson, A., Mole, L. E., and Porter, R. R. (1970), *Biochem. J. 116*, 249.

Gray, W. R. (1967), *Methods Enzymol. 2*, 469.

Hermodson, M. A., Ericsson, L. H., Titani, K., Neurath, H., and Walsh, K. A. (1972), *Biochemistry 11*, 4493.

Hurst, M. M., Niedermeier, W., Zikan, J., and Bennett, J. C. (1973), *J. Immunol. 110*, 840.

Kehoe, J. M., and Capra, J. D. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 2019.

Klotz, I. M. (1967), *Methods Enzymol. 11*, 576.

Köhler, H., Shimizu, A., Paul, C., Moore, V., and Putnam, F. W. (1970), *Nature (London) 227*, 1318.

Metzger, H. (1970), *Advan. Immunol. 12*, 57.

Migita, S., and Putnam, F. W. (1963), *J. Exp. Med. 117*, 81.

Moore, V., and Putnam, F. W. (1973), *Biochemistry 12*, 2361.

Pisano, J. J., and Bronzent, T. J. (1969), *J. Biol. Chem. 244*, 5597.

Plaut, A. G., and Tomasi, T. B., Jr. (1970), *Proc. Nat. Acad. Sci. U. S. 65*, 318.

Ponstingl, H., and Hilschmann, N. (1972), *Hoppe-Seyler's Z. Physiol. Chem. 353*, 1369.

Porter, R. R. (1973), *Science 180*, 713.

Press, E. M., and Hogg, N. M. (1970), *Biochem. J. 117*, 641.

Putnam, F. W. (1969), *Science 163*, 633.

Putnam, F. W. (1970), *in* Homologies in Enzymes and Metabolic Pathways and Metabolic Alterations in Cancer, Whelan, W. J., and Schultz, J., Ed., Amsterdam, North-Holland Publishing Co., p 361.

Putnam, F. W., Florent, G., Paul, C., Shinoda, T., and Shimizu, A. (1973), *Science 182*, 287.

Putnam, F. W., Kozuru, M., and Easley, C. W. (1967), *J. Biol. Chem. 242*, 2435.

Putnam, F. W., Low, T., Liu, V., Huser, H., Raff, E., Wong, F. C., and Clamp, J. R. (1974), *in* International Symposium on IgA, Kraus, F., and Mestecky, J., Ed., New York, N. Y., Plenum Publishing Co. (in press).

Putnam, F. W., Shimizu, A., Paul, C., and Shinoda, T. (1972), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 31*, 193.

Putnam, F. W., Shimizu, A., Paul, C., Shinoda, T., and Köhler, H. (1971), *Ann. N. Y. Acad. Sci. 190*, 83.

Ray, A., and Cebra, J. J. (1972), *Biochemistry 11*, 3647.

Shimizu, A., Putnam, F. W., Paul, C., Clamp, J. R., and Johnson, I. (1971), *Nature (London), New Biol. 231*, 73.

Titani, K., Whitley, E. J., Jr., and Putnam, F. W. (1969), *J. Biol. Chem. 244*, 3521.

Titani, K., Wikler, M., Shinoda, T., and Putnam, F. W. (1970), *J. Biol. Chem. 245*, 2171.

Wang, A. C., Fudenberg, H. H., and Pink, J. R. L. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 1143.

Wang, A. C., Gergely, J., and Fudenberg, H. H. (1973), *Biochemistry 12*, 528.

Wang, A. C., Pink, J. R. L., Fudenberg, H. H., and Ohms, J. (1970), *Proc. Nat. Acad. Sci. U. S. 66*, 657.

Witkop, B. (1968), *Science 162*, 318.

Woods, K. R., and Wang, K. T. (1967), *Biochim. Biophys. Acta 133*, 369.