H CHAIN SUBGROUPS OF MYELOMA PROTEINS AND NORMAL 7S γ-GLOBULIN*

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The immunoglobulins are structurally related, yet heterogeneous proteins that consist of at least two pairs of polypeptide chains, H(A) and L(B) that are linked by disulfide bonds (1, 2). The three major classes of immunoglobulins, $7S\gamma$, 19S, and β_{2A} , are each characterized by antigenically distinct H chains. Each class of immunoglobulin can be further divided into two major antigenic groups I and II, based on structural heterogeneity located on the L chains (3, 4). The present study was carried out in an attempt to clarify additional heterogeneity of γ -globulin observed with certain antisera. For this purpose, antisera to individual myeloma proteins proved most useful and the experiments performed indicated that human $7S\gamma$ -globulin consists of at least three subgroups based on antigenic heterogeneity of the H chains. A preliminary report of these findings has been presented (5).

Materials and Methods

Antigens and Antisera.—Myeloma proteins were isolated from patients' sera by means of starch block zone electrophoresis (6). New Zealand albino rabbits were immunized with several injections of 2 to 5 mg of the isolated myeloma protein or normal γ -globulin (Lederle fraction II), incorporated into complete Freund's adjuvant (Difco Laboratories, Inc., Detroit). Immunization was continued for 2 to 6 months and bleedings were obtained 2 to 4 weeks following a given injection starting at 2 months after the initial injection. The myeloma proteins used for immunization along with some of their characteristics were as follows:

Antigen	Electrophoretic mobility	Antigenic class	L chain type	
Vi	Slow	$7S\gamma$		
Fe	Fast	$7S\gamma$	I	
Ap	Slow	$7S_{\gamma}$	I	
Sp	Slow	7S ₇	II	
Ĉi	Slow	7Sγ	I	
Ge	Fast	$7\mathrm{S}_{\gamma}$	I	
Zu	Fast	$7S_{\gamma}$	No L chains*	
Ro	Fast	$7S_{\gamma}$	I	
We	Slow	$7S_{\gamma}$	1	
Ke	Fast	7S _Y	I	

^{* 50,000} molecular weight protein immunologically related to H chains and F fragment (7, 8). Antiserum to this protein was produced in a cynamologous monkey.

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Immunologic Techniques.—Ouchterlony agar diffusion (9), microimmunoelectrophoresis (10), and quantitative precipitin analysis (11), were performed as previously described. Protein concentrations were determined by the Folin procedure (12).

Reductive Cleavage of γ -Globulin.—L(A) and H(B) chains of Fr II and individual myeloma proteins were produced as previously described (13). Sixty mg of protein in 0.55 m tris, pH 8.2, was reduced for 1 hour at room temperature with 0.2 m 2-mercaptoethanol. Alkylation with 0.3 m iodoacetamide proceeded in the cold for 1 hour, and was followed by overnight dialysis against 1 m propionic acid. Separation of L and H chains was accomplished by means of a 2 x 55 cm sephadex G-100 column equilibrated with 1 m propionic acid. Isolated chains were dialyzed against several changes of distilled water and concentrated by means of pressure dialysis.

Enzymatic Cleavage of γ -Globulin.—F and S fragments of myeloma proteins were produced by papain digestion (14) using a protein to enzyme ratio of 100:1. Digestion was carried out in the presence of 0.01 m cysteine and 0.002 m EDTA, pH 7.5. Digestion was allowed to proceed for 16 hours at 37°C and was stopped by removal of cysteine by dialysis against a large volume of cold saline. Pepsin digestion (15) was performed using a 100:1 protein to enzyme ratio at pH 4.0. Digestion continued for 16 hours at 37°C and was stopped by dialysis against a large volume of 0.1 m phosphate buffer, pH 7.5. Reduction of the 5S fragment produced by pepsin digestion was performed overnight at 37°C using 0.01 to 0.1 m 2-mercaptoethanol. When air reoxidation was attempted, the reducing agent was removed by passing 1 ml of the protein solution through a 1 x 30 sephadex G-25 column. The protein solution was allowed to stand overnight at room temperature in an atmosphere of 100 per cent oxygen. Analytic ultracentrifugation of digestion products before and after reoxidation was performed in a Spinco model E ultracentrifuge equipped with schlieren optics.

RESULTS

Characterization of Vi Subgroup of Myeloma Proteins.—The procedure used to divide myeloma proteins of the 7S γ -globulin class into subgroups was based on the ability of antisera to different myeloma proteins to react to varying degrees with a battery of other isolated myeloma proteins. Of a large number of antisera tested, six were most useful in differentiating the myeloma proteins into subgroups. Table I summarizes the results obtained by Ouchterlony plate analysis with these antisera after they had been absorbed with selected heterologous myeloma proteins. After absorption, all antisera showed some degree of specificity for the individual myeloma protein used for immunization. In addition, certain other myeloma proteins were capable of reacting with these antisera, while others could not. The reactions of thirteen selected myeloma proteins with these antisera are shown in Table I. On the basis of this analysis, three subgroups of myeloma proteins were differentiated: a Vi subgroup first differentiated by an anti-Vi rabbit antiserum, a Ge subgroup, and a major group of myeloma proteins which consisted of 60 to 70 per cent of all myeloma proteins tested. There was also a less well defined group of myeloma proteins which were clearly distinct from the above three groups. They have been omitted from the table because further work is required for their delineation.

Fig. 1 illustrates the reaction of the absorbed anti-Vi antiserum with Vi

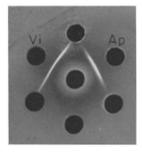
protein, three of the related myeloma proteins of the Vi subgroup, and thirteen unrelated proteins. Strong reactions occurred with the myeloma proteins of the Vi group while the remainder of myeloma proteins gave no reaction. With un-

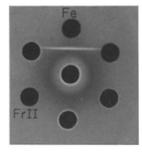
TABLE I

The Reaction of Various Myeloma Proteins with Different Absorbed Antisera Bringing Out

Three Classes of 7S Myeloma Proteins

Myeloma protein	Anti-Vi abs. with Sp	Anti-Fe abs. with Ke	Anti-Sp abs. with Vi	Anti-Ci abs. with We	Anti-Ge abs. with We	Anti-Zu abs. with Ke
Vi	+++	++	0	0	0	++
\mathbf{Fe}	++	+++	0	0	0	++
$\mathbf{A}\mathbf{p}$	++	++	0	0	0	++
We	0		++	0	0	0
Gr	0	0	++	0	0	
Le	0	0	++	0	0	0
Ku	0	0	++	0	0	0
Ke	0	0	++	0	0	0
Cu	0	0	++	0	0	0
\mathbf{Br}	0	0	++	0	0	0
Ge	0	0	0	++	++++	0
Ci	0	0	0	++++	++	0
Ro	0	0	0	++	+	0
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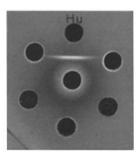
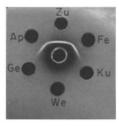


Fig. 1. Illustration of the specific reaction of myeloma proteins of the Vi subgroup (Vi, Ap, Fe, Hu) with absorbed anti-Vi antiserum. Fr II and thirteen other myeloma proteins in peripheral wells failed to react. The three central wells contain the antiserum.

absorbed antisera the Vi group spurred over the heterologous proteins. Anti-Fe antiserum, made against another of the myeloma proteins of the Vi subgroup, after absorption reacted only with the Vi subgroup of myeloma proteins as indicated in column 2 of Table I. An antiserum to a heterologous myeloma

protein Sp showed the Vi subgroup of myeloma proteins plus those in the Ge subgroup to be deficient antigenically relative to other myeloma proteins. Finally, a monkey anti-Zu antiserum, after absorption with an unrelated myeloma protein, still reacted with the myeloma proteins of the Vi subgroup to the exclusion of all other myeloma proteins tested as shown in Fig. 2. The Zu



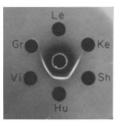


Fig. 2. Reaction of Zu protein and five proteins of the Vi subgroup with monkey anti-Zu antiserum absorbed with a heterologous myeloma protein. Six heterologous myeloma proteins, tested in the other peripheral wells, failed to react.

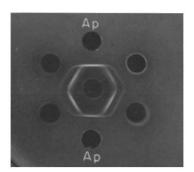


Fig. 3. Reaction of the γ -globulin of four different normal human sera and a myeloma protein of the Vi subgroup (Ap) with unabsorbed rabbit anti-Vi antiserum. All normal sera demonstrated two precipitin bands with the Vi antiserum, a thick inner band and thin outer band. The line formed by the Vi protein spurred over the inner band and formed a line of identity with the outer band.

protein, present in large quantities in the serum of the same patient, was a low molecular weight protein immunologically related to H chains and the F fragment of γ -globulin and, as shown in Fig. 2, it showed a close relationship to the Vi subgroup of myeloma proteins when the monkey antiserum was used.

A total of 64 myeloma proteins were tested with these antisera and ten were found to belong to the Vi subgroup. All ten of these myeloma proteins showed a reaction of identity with both the rabbit and the monkey antisera.

Relation of Vi Subgroup to Normal γ -Globulin.—When Vi antiserum was reacted in the unabsorbed state with normal γ -globulin or with the γ -globulin of

individual normal human sera, as shown in Fig. 3, two lines of precipitation resulted: a thick band close to the antiserum well and a thin outer band. The precipitin band formed by the myeloma protein of the Vi subgroup placed in the adjacent wells spurred over the inner band and gave a line of identity with the outer band. In order to show this relationship, the myeloma protein was

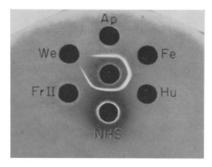


Fig. 4. Reaction of Vi subgroup myeloma proteins (1 mg/cc) and normal γ -globulin (10 mg/cc) with rabbit anti-Vi antiserum absorbed with a heterologous myeloma protein. A single line of identity was formed between the Vi subgroup of myeloma proteins and the portion of normal γ -globulin containing the Vi specificity. A heterologous myeloma protein (We) gave no reaction.

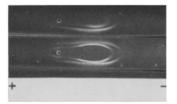


Fig. 5. Immunoelectrophoretic distribution of the Vi subgroup in normal γ -globulin. Two normal sera were placed in wells and unabsorbed anti-Vi antiserum in the trough. Heavy band close to the trough results from the reaction between non-Vi-specific γ -globulin and antibody while the inner line represents the reaction between the Vi subgroup of normal γ -globulin and antibody specific for this subgroup. The lower serum contains a higher concentration of Vi type protein.

used at a concentration of 1 mg/cc while the normal γ -globulin was at 10 mg/cc Fig. 4 shows the reaction of Vi antiserum after absorption with a heterologous myeloma protein with the Vi subgroup of myeloma proteins and normal γ -globulin. Here, a single line of identity was formed between the myeloma proteins and a constituent of normal human γ -globulin. These data indicated that a portion of the population of normal γ -globulin molecules contained the antigenic determinants specific to the Vi subgroup. Fig. 5 shows the electrophoretic distribution of the Vi related portion of normal γ -globulin. The lines

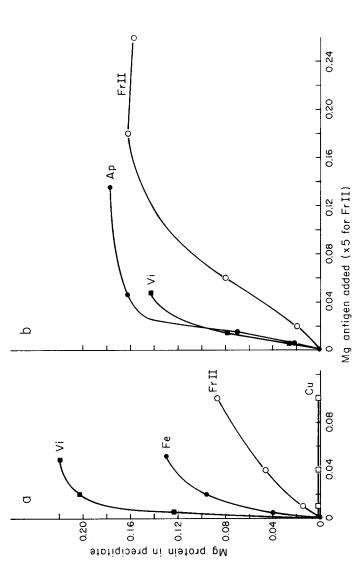


Fig. 6. Quantitative precipitin analysis of Fr II and myeloma proteins with absorbed rabbit anti-Vi antiserum (a); and absorbed monkey anti-Zu antiserum (b).

(a) Maximum quantity of antibody was precipitated by the antigen which elicited the immune response, Vi, due to individual specificity. A Vi subgroup protein Fe, and Fr II precipitated lesser amounts of antibody. A heterologous protein Cu gave no precipitation.
(b) Fr II and two Vi subgroup myeloma proteins all precipitated approximately equal amounts of an absorbed monkey anti-Zu antiserum. In both (a) and (b) considerably more Fr II than myeloma protein was needed in order to precipitate a comparable amount of antibody.

closest to the antiserum trough represent the reaction of the bulk of γ -globulin with the non–group-specific antibodies of the Vi antiserum, and the short lines behind it, the reaction of the Vi related portion with the Vi group-specific antibody. The average mobility of both lines appeared to be approximately the same.

In order to estimate the relative amount of the Vi subgroup present in Fr II, quantitative precipitin analysis was performed using rabbit anti-Vi antiserum and monkey anti-Zu antiserum, both absorbed with a heterologous myeloma protein, as shown in Fig. 6. The difference in the amount of precipitate formed

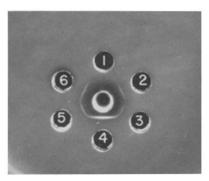
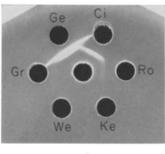


Fig. 7. Localization of specificity for Vi subgroup on 5S pepsin fragment. Center well, rabbit anti-Vi antiserum absorbed with heterologous myeloma protein. Well *I*, reduced and alkylated Vi subgroup myeloma protein (Fe); 2 and 3, reduced and reoxidized pepsin treated Fe; 4, unreduced 5S pepsin fragment from Fe protein; 5, untreated Fe protein; 6, four parts reduced and alkylated Fe protein and one part untreated Fe protein. Antigenic specificity is contained in the pepsin 5S fragment and lost upon reduction.

with the Vi protein and Fe protein and anti-Vi antiserum was due to antibody present in the Vi antiserum that was specific for the eliciting antigen Vi. In Fig. 6 a the equivalence zone was not quite reached for Fr II or Fe protein, while the unrelated myeloma protein gave no precipitation with the Vi antiserum. On the basis of a comparison of the antigen-antibody ratios in antibody excess in these and similar experiments, the Vi-related proteins were 10 to 50 times as efficient in precipitating the anti-Vi antiserum as Fr II. If the valency and precipitating capacity of the myeloma protein and the reactive molecules of Fr II were the same, these experiments would indicate that 2 to 10 per cent of Fr II was made up of molecules of the Vi subgroup. Quantitative precipitin analysis with normal γ -globulin from twenty individual sera and the anti-Vi antiserum showed the quantity of Vi type protein present in these normal sera to vary from 0.25 to 1.1 mg/ml.

Localization of Antigenic Specificity of Vi Subgroup.—Experiments were performed to determine the portion of the γ -globulin molecule that contained the

antigenic specificity of the Vi subgroup. For this purpose the monkey anti-Zu and the rabbit anti-Vi antisera were used to test papain- and pepsin-digested myeloma proteins of the Vi group. Immunoelectrophoretic experiments using the monkey anti-Zu antiserum in the unabsorbed state, showed that it only reacted with the F component of either Fr II or various myeloma proteins. Following absorption with myeloma proteins of the major myeloma group or with limited amounts of Fr II, the F component of the Vi group myelomas continued to show reactivity by immunoelectrophoresis while the other myelomas lost their F precipitin line. Also, an isolated H chain preparation from protein Ap of the Vi subgroup was capable of reacting with the absorbed anti-Zu antiserum while no reaction occurred with isolated L chains.



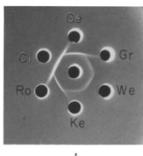


Fig. 8. Reactions between Ge subgroup of myeloma proteins and absorbed anti-Ge anti-serum (a), and unabsorbed anti-Ci antiserum (b).

- (a) Reaction of Ge and the two other Ge-related proteins (Ci, Ro) with anti-Ge antiserum absorbed with a heterologous myeloma protein.
- (b) Ci and two Ge-related proteins (Ge, Ro) spur over the precipitin bands formed by three heterologous myeloma proteins and anti-Ci antiserum. The spur of Ge in (a) and of Ci in (b) over the other related myeloma proteins is due to individual specificity of the antiserum for the antigen which elicited the immune response.

When similar experiments were performed with the rabbit anti-Vi antiserum, no precipitin line was obtained with either the F or S fragment. The antigenic specificity could be localized, however to the 5S fragment produced following pepsin digestion as shown in Fig. 7. When the 5S fragment or the undigested myeloma protein was reduced with 0.1 M 2-mercaptoethanol, all reactivity was lost. In addition, the reduced protein could not inhibit the precipitation of the unreduced protein when present together in the same well. The antigenic activity could be regained by reoxidation of the reduced material following removal of the reducing agent. These experiments indicate that part of the antigenicity of the Vi group is contained in the unreduced 5S pepsin S fragment and is dependent upon the integrity of one or more disulfide bonds for its antigenicity.

Ge Subgroup. As indicated in Table I the antisera to myeloma proteins were able to distinguish another subgroup of myeloma proteins which will be tentatively referred to as Ge. Rabbit anti-Ge antiserum after absorption with Bence Jones proteins of both groups and an unrelated myeloma protein was still capable of reacting with the myeloma proteins of the Ge subgroup as well as a small fraction present in normal human serum. Fig. 8 a shows the reaction of an absorbed anti-Ge antiserum with Ge, two other related myeloma proteins, and three unrelated myeloma proteins, and Fig. 8 b shows the reaction of an unabsorbed anti-Ci antiserum with the same proteins. The strong spur of Ge and Ci over the related proteins when tested with the homologous antiserum, was due to the reaction involving antigenic determinants specific for the immunizing antigen. Three myeloma proteins of 50 tested were classified in the Ge subgroup.

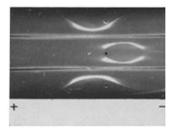


Fig. 9. Unique electrophoretic mobility of the F fragment of Ge subgroup. Upper well, Ge protein; middle well, a heterologous myeloma protein; lower well, Ci protein. Rabbit anti-Fr II F fragment was placed in the troughs.

It was possible to localize some of the antigenic determinants which distinguish the Ge subgroup from the rest of Fr II. When an antiserum to the heterologous myeloma protein Sp was absorbed with a protein of the Ge subgroup, it was still capable of precipitating with the majority of myeloma proteins tested. When the papain digests of these proteins were examined, only the F fragment of the protein reacted with the absorbed antiserum. This demonstrates that some of the antigenic determinants present on the heterologous myeloma proteins that distinguish them from the Ge subgroup are present on the F fragment.

Unique Character of F Component of Ge Subgroup.—When immunoelectrophoresis was performed using the Ge antiserum and Fr II two bands were formed. The band referable to the portion of molecules specific for the Ge subgroup had a slightly faster mobility than that formed with the bulk of Fr II. Also, two of the three myeloma proteins in this group were the two fastest migrating of 35 myelomas examined by immunoelectrophoresis and starch gel electrophoresis and the third was of average mobility. When the papain digests of the Ge subgroup were compared to those of other myeloma proteins a striking

difference in the mobility of the F fragments was observed (Fig. 9). All three proteins of the Ge subgroup had faster migrating F components than any of the myeloma proteins tested. The S fragments on the other hand varied from one myeloma protein to the next and were in general related to the electrophoretic mobility of the whole protein. On the other hand, the mobility of the F fragment of the Vi subgroup and the major group of myeloma protein was with only minor differences detected by starch gel electrophoresis.

DISCUSSION

The classification and subdivisions of γ -myeloma proteins and 7S γ -globulin are based primarily on antigenic differences that may be summarized as follows: (a) antigenic determinants present on the H chains and F fragment that distinguish 7S γ -globulin from the two other major groups of immunoglobulins, 19S and β_{2A} (16); (b) the major antigenic groups I and II present on the L chains (3, 4); (c) subdivision of γ -globulins based on antigenic heterogeneity within the major L chain groups (17); (d) subdivision of γ -globulins based on heterogeneity within the major H chain groups; (e) specific antigenic determinants which appear to be unique to an individual myeloma protein or antibody (18-20).

The experiments reported above demonstrate subdivision of 7S γ -globulin based on antigenic heterogeneity of H chains and should be distinguished from antigenic heterogeneity of L chains as well as the individual antigenic specificity. The data indicating that the Vi subgroup is based on differences located on the H chains are as follows: (a) the Vi subgroup contained two group II and eight group I myeloma proteins. Since the two major L chain groups do not share common antigens, any subdivision of γ -globulin which brings both group I and II myelomas together must have something other than L chains as the common antigen; (b) after absorption with an appropriate myeloma protein, isolated L chains of a myeloma from one of the subgroups failed to react with the antiserum whereas the whole myeloma protein did; (c) with the monkey antiserum the antigenic specificity of the Vi subgroup was localized to the F fragment of the H chains produced by papain; (d) antisera to the major group of myeloma proteins recognized the Vi group as deficient and this deficiency was also localized to the F fragment; (e) with the monkey anti-Zu antiserum, it has been possible to identify the antigenic subgroup specificity on isolated H chains.

In contrast to the above subdivision based on H chain heterogeneity, previous studies on the antigenic structure of Bence Jones proteins have revealed differences in these proteins that subdivide group I and group II L chains (17). The subgrouping of 7S γ -globulin reported in the present paper must also be distinguished from the antigen-antibody reaction which is specific for the individual myeloma protein against which the antiserum was made. Whereas 5 to 10 mg of Fr II per cc of antiserum removed all antibody from the Ge and Vi

antisera that was capable of precipitating the myeloma proteins of the appropriate subgroup, strong precipitin bands remained when these antisera were reacted with the homologous antigens, Ge and Vi. Also, Ouchterlony and quantitative precipitin analysis showed that after absorption with 50 to 75 mg of Fr II less than half the antibody specific for the individual myeloma protein was removed.

Utilizing monkey antisera to normal human γ -globulin, others (21, 22) have been able to distinguish three lines by immunoelectrophoresis of human 7S γ -globulin. Preliminary evidence has been presented recently (22) that these differences were localized to the F fragment. It remains to be determined whether these variations are related to the groups described in the present study.

The finding that part of the antigenic specificity of the Vi subgroup was located on the 5S fragment produced by pepsin digestion and not on the 3.5S fragments produced by papain or by reduction of the 5S pepsin fragment indicates the importance of disulfide bonds in maintaining the necessary configuration for the antigenic specificity observed with the rabbit antisera. It seems likely that the disulfide bond that holds the 3.5S fragments together is the crucial bond for the maintenance of these antigenic determinants, although the possible role of intra H chain disulfide bonds is not excluded. Air reoxidation of the papain S fragments failed to produce any 5S material nor did the antigenic specificity of the Vi subgroup reappear. This suggests that the papain and pepsin S fragments of the Vi subgroup differ structurally and that the disulfide bonds necessary for part of the antigenic specificity of this subgroup are either not present on the papain S or are not available for reoxidation following reduction, whereas they are present and are readily oxidized following reduction of the S fragment produced by pepsin digestion. The monkey antiserum, however, which distinguished the Vi subgroup in a completely analogous fashion reacted solely with the F fragment. Thus the two types of antisera which recognize the Vi subgroup react with very different determinants. This was also brought out by the fact that the Zu protein from the patient with H chain disease failed to react with the rabbit anti-Vi antiserum, but with the monkey anti-Zu antiserum the Vi group and Zu protein showed the same specific antigenic determinants.

In the present study it was shown that some of the differences between the Ge subgroup and other myeloma proteins reside in the F fragment. In addition through the use of antisera to the S fragment of Fr II absorbed with light chains, the myeloma proteins of the Ge subgroup appeared to be antigenically deficient in this portion of the H chain when compared to myeloma proteins of the other two groups (23). This would suggest that the antigenic heterogeneity of this subgroup also extends over a major portion of the H chain and is not limited to the F fragment alone.

The finding of a difference in mobility of the F fragment of the Ge subgroup

compared to that of other myeloma proteins, is of considerable interest. The two most likely explanations of the fast mobility which are at present under investigation are: (a) increase in the carbohydrate moiety of the F fragment, especially sialic acid; and (b) increase in the number of basic amino acids in the F fragment of this subgroup.

The exact incidence of myeloma proteins in the Vi and Ge subgroups remains to be determined. The best figures are available for the Vi group which was 15 per cent. This is slightly higher than the figure obtained for the concentration of this type of protein in Fr II γ -globulin. However, since considerable variation in the quantity of this protein was shown to exist in individual sera, further quantitative studies with different normal sera are necessary to settle this point. Previous studies showed a good correlation between the relative incidence of myeloma proteins of a given antigenic class or group with the known percentage of normal γ -globulin molecules that were antigenically related to the myeloma protein (3).

Most of the above studies involved the three clearly definable subgroups, Vi, We, and Ge. However, in addition, a number of other myeloma proteins were encountered which did not fit into any of the three categories. These represent proteins of at least one other subgroup and are currently under investigation.

Since the various groups described reflect differences in the H chains, it naturally became of interest to relate the groups to the Gm system of genetic types of γ -globulin which is known to involve the H chains. Such studies, carried out in collaboration with Drs. Mårtensson and Grubb, will be published separately (24). They have shown some surprising results. Only myeloma proteins of the Vi class were Gm(b+); all proteins of the other classes were Gm(b-). Proteins of the Ge subgroup were negative for all the Gm factors. The proteins of the major group in Table I were either Gm(a+) or Gm(f+). It became apparent that consideration will have to be given to the subgroups for a proper understanding of the genetic systems involving γ -globulin.

SUMMARY

Through the use of a variety of antisera to isolated myeloma proteins, four subgroups of 7S γ -globulin type proteins were readily distinguished. The first, the Vi subgroup, consisted of ten of 64 myeloma proteins studied. The second, the We group, contained the majority of myeloma proteins. The third, the Ge subgroup, included three of 50 myeloma proteins. The fourth remains ill-defined and appears heterogeneous.

Counterparts for both the Vi and the Ge subgroup, were found in the Fr II γ -globulin and in the normal γ -globulin of all of a large number of individual sera studied.

The unique antigenic character of both groups was localized to the H chains, although different determinants were involved for different antisera. An essential role of intact disulfide bonds was apparent with certain rabbit antisera.

In addition to the special antigenic characteristics, the Ge subgroup showed in each instance a fast mobility for the F fragments produced by papain which was not found for other myeloma proteins.

Note Added in Proof.—Exchange of myeloma proteins with Dr. W. Terry and Dr. J. Fahey indicates that the Vi subgroup is the same as the γ_{2o} -subgroup of these authors. The We subgroup corresponds to γ_{2b} . The Ge subgroup was not related to any of the subgroups described by these authors.

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