INDIVIDUAL ANTIGENIC SPECIFICITY OF MYELOMA PROTEINS

CHARACTERISTICS AND LOCALIZATION TO SUBUNITS*

BY HOWARD M. GREY, M.D., MART MANNIK, M.D., AND HENRY G. KUNKEL, M.D.

(From The Rockefeller Institute)

(Received for publication, January 12, 1965)

The homogeneous character of the myeloma proteins has served as a useful model in furthering the understanding of the heterogeneous nature of normal γ-globulin. Studies on the antigenic structure of myeloma proteins led in considerable part to the classification of the immunoglobulins into the major classes γG, γA, and γM[1] (1), as well as into the two major light chain groups K and L[1] (2–3), and more recently to a division of the γG-class into several subgroups based on heavy chain heterogeneity (4–6). Along with antigenic determinants which are shared by other proteins of the same class, group, or subgroup, individual myeloma proteins contain antigenic determinants which appear to be characteristic of the myeloma protein and are not found on other myeloma proteins (7–9). Similar individual antigenic specificity has also been found in a number of purified antibody preparations (10, 11).

The purpose of the present experiments was to determine the site or sites on the myeloma protein to which this individual specificity was localized through a study of various subunits obtained by reductive and enzymatic cleavage of these proteins. The likely relevance of such investigations to the question of the antigenic and structural individuality of antibodies represented a major reason for this analysis. In addition, further studies were carried out on the relationship of the individual specificity to the various subgroups of γ-globulin and to minor populations of molecules in pooled γ-globulin.

Materials and Methods

Multiple Myeloma Proteins and Bence Jones Proteins.—Sera were obtained from patients with an established diagnosis of multiple myeloma. The myeloma proteins (abbreviated MM protein in illustrations) were isolated from other serum components by zone electrophoresis

* Aided by a grant from the Public Health Service No. AM04761-04.

[1] The nomenclature used in this report for the immunoglobulins and their subunits will follow the recommendation of the World Health Organization committee on Nomenclature of Human Immunoglobulins. Thus γG = 7Sγ, γA = β_2A, γM = 19Sγ, group K = group I and group L = group II.

on starch medium (12). Urine was collected from the same patients, and Bence Jones proteins, if present, were isolated as previously described (2). The presence of classical Bence Jones proteinuria was established by the heat test after adjusting the urine pH to 4.9 ± 0.1. Pooled human fraction II (Lederle Laboratories Pearl River, New York was used as the source for normal γ-globulin.

Antisera.—Antisera to Bence Jones proteins and serum myeloma proteins were produced by immunizing rabbits with water-in-oil emulsions of the isolated proteins and complete Freund's adjuvant (Difco Laboratories, Inc., Detroit). Multiple injections of 2 to 3 mg were given subcutaneously over a 3 to 6 month period. In order to prepare specific antisera to individual myeloma proteins, antisera were absorbed with sufficient normal γ-globulin (Fr II) so that they reacted only with the homologous myeloma proteins and not with Fr II or other myeloma proteins when tested in gel diffusion experiments at a concentration of 1 mg/cc. The amount of Fr II needed for absorption varied from 1 to 10 mg/ml of antiserum, depending on the antiserum to be absorbed.

Immunologic Techniques.—Ouchterlony agar diffusion (13), microimmunoelectrophoresis (14), and quantitative precipitin analysis (15), were performed as previously described. Protein concentrations were determined by the Folin procedure (16).

Reductive Cleavage of γ-Globulin.—Light and heavy chains of individual myeloma proteins were produced as previously described (17). 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 light and heavy chains was accomplished by means of a 3 x 55 cm Sephadex G-100 column equilibrated with 1 M propionic acid. Only the descending portion of the first peak was used for the heavy chain preparation. Isolated chains were dialyzed against several changes of distilled water and concentrated by means of vacuum dialysis. Chains were recombined in the weight ratio 5H: 1L or 2H: 1L at neutral pH. Recombined chains were incubated for 24 hours at 4°C before being used for immunologic studies.

Enzymatic Cleavage of γ-Globulin.—Fc and Fab fragments[2] of myeloma proteins were produced by papain digestion (18) 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.

RESULTS

Effect of Absorption with Fr II on Antisera Specific for Individual Myeloma Proteins.—In order to determine whether Fr II contained molecules with the same antigenic specificity of an individual myeloma protein, the following experiments were carried out. Antisera to individual myeloma proteins were screened for the presence of antibodies individually specific for the homologous myeloma protein. These were found in the vast majority of instances. The criterion used was the presence of a spur when the antiserum was tested against the homologous protein, Fr II, and other myeloma proteins in gel diffusion at the same protein concentration. The positive antisera were then absorbed with

[2] The Fc fragment refers to the papain digestion product previously called the "fast" fragment and the Fab fragment refers to what was previously called "slow" fragment. The Fd fragment designates the heavy chain portion of the Fab fragment, previously called the "A-piece."

various concentrations of Fr II γ-globulin (2- to 100 mg/ml) and then tested with the homologous myeloma protein by quantitative precipitin analysis. The dilution effect caused by the absorbant Fr II and non-specific precipitation was corrected for through the use of appropriate control antisera. Fig. 1 represents the three types of results encountered. Fig. 1 *a* demonstrates the virtually complete removal of antibodies specific for the homologous myeloma protein. Only

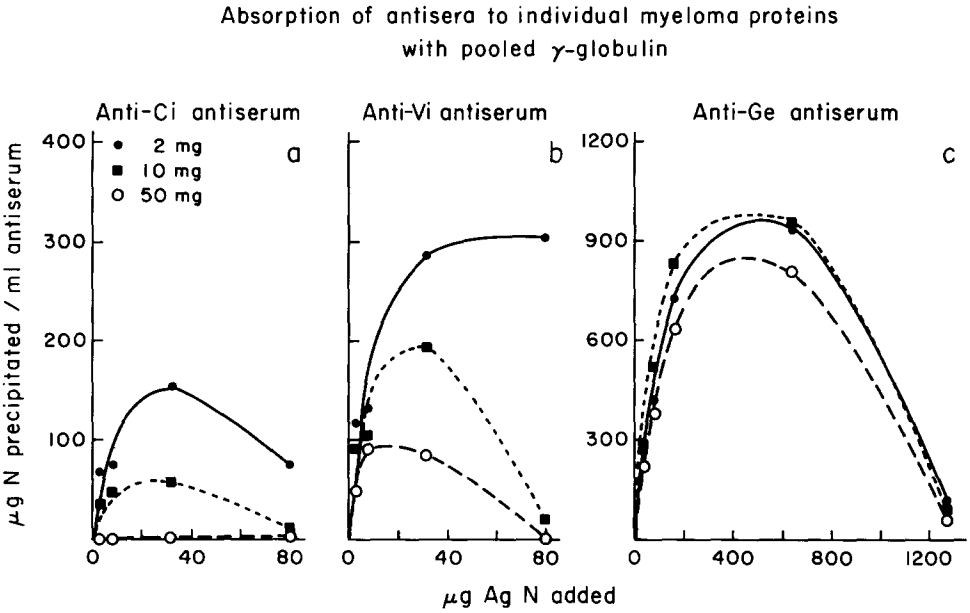

FIG. 1. Quantitative precipitin analysis of rabbit anti-myeloma protein antisera, reacted with the homologous myeloma protein after absorption with 2, 10, and 50 mg of pooled γ-globulin. The curves shown are representative examples of the three patterns observed; (*a*) complete loss of individually specific antibody on absorption with normal γ-globulin, (*b*) partial absorption of antibodies specific for the homologous myeloma protein, (*c*) little or no loss of antibodies specific for the homologous myeloma protein after absorption with normal γ-globulin.

partial absorption of individually specific antibodies was possible with the antiserum used in Fig. 1 *b*, and only slight loss of individual specificity was observed after absorption of the antiserum used in Fig. 1 *c*. Of six antisera tested two showed complete removal of individual specificity, three showed partial removal and one showed only slight removal of antibodies specific for the homologous protein. In general the antisera with the highest titer of anti-γ-globulin antibodies showed the greatest degree of individual specificity remaining after absorption with 50 mg of Fr II as in Fig. 1, but exceptions were encountered. Anti-Ge antiserum was also absorbed with 100 mg of Fr II per cc and with

pooled concentrated normal serum but marked individual specificity remained; however agar gel analysis indicated reduction in the intensity of the precipitin bands after such absorption.

An important consideration in evaluating the individual specificity was the heavy chain subgroup to which the myeloma protein belonged. Various amounts of Fr II were required to remove these subgroup specific antibodies and much of the initial depletion in the precipitin curve represented this factor rather than true depletion of individual specificity. For example, in Fig. 1 *b* the initial absorption with 2 mg of Fr II per cc removed the reactivity of this antiserum with all myeloma proteins except those belonging to the Vi subgroup, approximately 10 per cent of myeloma proteins (4). However, it took 10 mg of Fr II to remove the subgroup reactivity which then left the antiserum specific for the individual myeloma protein. Thus, much of the fall in the curve in Fig. 1 *b* can be explained

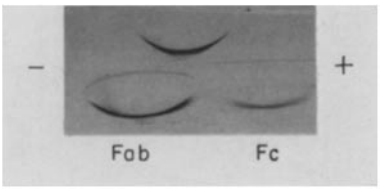

FIG. 2. Localization of individual specificity to the Fab fragment produced by papain digestion. Upper well, unsplit Ge myeloma protein; lower well, papain digested Ge myeloma protein; middle trough, rabbit anti-Ge antiserum absorbed with 10 mg Fr II; lower trough, anti-Ge antiserum unabsorbed. The Fab fragment reacted with the absorbed antiserum whereas the Fc fragment did not.

on the basis of removal of antibodies to the minor subgroup and not to a true depletion of the individual specificity. The experience with most antisera indicated that antibodies to both heavy and light chain subgroups were an important factor in the initial fall of the precipitin curves after absorption with γ-globulin so that subgroup specificity had to be differentiated from true individual specificity. Agar plate analyses of the antigens and absorbed antisera that showed individual specificity indicated that in a number of instances multiple lines were obtained. This suggested that these antisera were detecting heterogeneity within the myeloma proteins used as antigen. Preliminary efforts to relate this heterogeneity to the known fine banding of many myeloma proteins in starch gel systems (19) were unsuccessful; however, considerable difficulty was encountered in isolating these bands for antigenic analysis.

Localization of Individual Specificity on Papain Fragments.—Papain digestion was carried out on nine myeloma proteins whose antisera demonstrated individual specificity. Immunoelectrophoretic analysis was performed on the papain-split myeloma proteins and in all cases it was possible to localize the individual specificity of the myeloma protein to the Fab fragment as illustrated

in Fig. 2 for the anti-Ge antiserum. Similar results had been obtained for the individual specificity of isolated antibodies (10).

Localization of Individual Specificity to Light and Heavy Polypeptide Chains.— Light and heavy chains were separated and tested for purity with specific anti-light chain and anti–heavy chain antisera by gel diffusion. The localization of individual specificity was then determined by testing the separate and recombined chains with the specific antiserum before and after absorption with Fr II. Figs. 3, 4, and 5 illustrate typical results obtained. Fig. 3 demonstrates the presence of individual specificity on the light chains of myeloma protein Sz. The light chain reactivity accounted for all the specificity as evidenced by the line

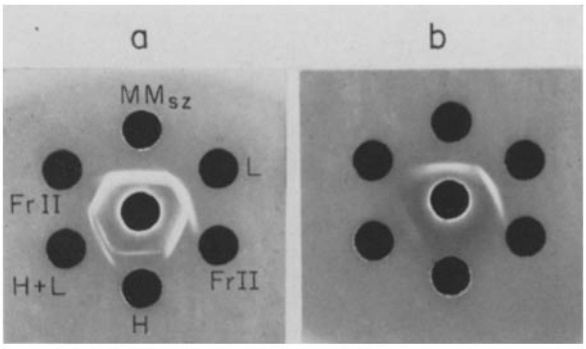

FIG. 3. Reaction of the Sz protein and polypeptide chains with individually specific rabbit anti-Sz antiserum before and after absorption with Fr II. (*a*) The whole protein, isolated light chains, (L) and recombined chain (H and L) spurred over Fr II when unabsorbed antiserum was used. (*b*) After the antiserum was absorbed with 2 mg Fr II the whole protein and the isolated light chains gave a reaction of identity and thereby localized the individual specificity of the Sz protein to the light chains. The recombined chains also showed a precipitin band whereas isolated heavy chains and Fr II failed to react.

of identity formed between the whole myeloma protein and the light chain preparation when tested with the absorbed antiserum. The double band formed by the recombined chains with the unabsorbed antiserum is due to an excess of heavy chains. Fig. 4 illustrates an example where the individual specificity was localized to the heavy chains. The purity of the heavy chain preparation is shown by the spur of Fr II through the heavy chain band due to the reaction of the light chain determinants of Fr II with the unabsorbed anti-Ne antiserum. In other experiments it was shown that the heavy chains formed a line of identity with the whole myeloma protein when reacted with the absorbed anti-Ne antiserum thereby indicating that all the individual specificity of the whole protein was present on the isolated heavy chains.

The third type of pattern is shown in Fig. 5 which shows the reactions obtained with the Ro protein and its rabbit antiserum. In this case the individual

specificity was not present in either of the isolated chain preparations but was present when the chains were recombined. Fig. 5 *a* shows the reaction with the unabsorbed antiserum. The antigenic deficiency of the isolated chains is shown by the spur of Fr II over the heavy and light chains whereas the recombined chains formed a line of identity with the whole myeloma protein and spurred over Fr II. The inner band formed between the unabsorbed antiserum and the recombined chains was caused by an excess of light chains. The outer band represents the recombined chains. When the Ro antiserum was absorbed with Fr II only the whole myeloma protein and the recombined chains reacted as shown in Fig. 5 *b*. When the unabsorbed antiserum was reacted with the iso-

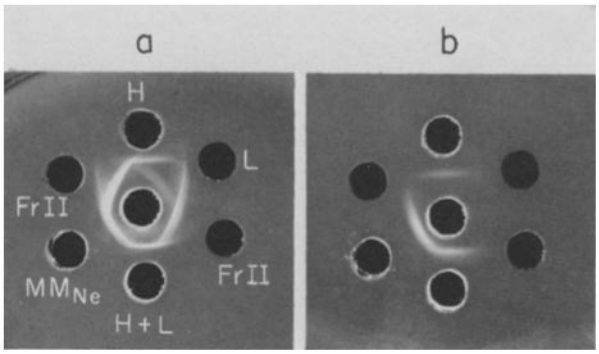

FIG. 4. Localization of individual specificity to the heavy chains of Ne myeloma protein. (*a*) With the unabsorbed antiserum the isolated heavy chains as well as the whole protein and recombined chains spurred over Fr II. The heavy chains (H) were antigenically pure as indicated by the spur of Fr. II through them due to a light chain anti-light chain reaction. (*b*) When the antiserum was absorbed with 2 mg of Fr II only the isolated heavy chains, whole protein and recombined chains reacted indicating the localization of individual specificity to the heavy chains.

lated Ro chains in adjacent wells, the reaction depicted in Fig. 5 *c* occurred. The two major bands formed between the isolated chains and the unabsorbed Ro antiserum showed the expected reaction of non-identity. A third irregular band, however, was also formed behind the intersection of the two bands. This was interpreted as representing the reaction between antibody which did not react with either of the isolated chains but that did react with the recombined chains. In this case sufficient recombination of heavy and light chains apparently occurred in the agar upon diffusion and admixture of the isolated chains. This third band which had a variety of irregular features in different experiments was the only one formed when the isolated chains were reacted with the Ro antiserum absorbed with Fr II. No inhibition of the precipitation of the whole myeloma protein occurred when either heavy or light chains were placed in wells adjacent to the whole protein. This indicated that the antigenic deter-

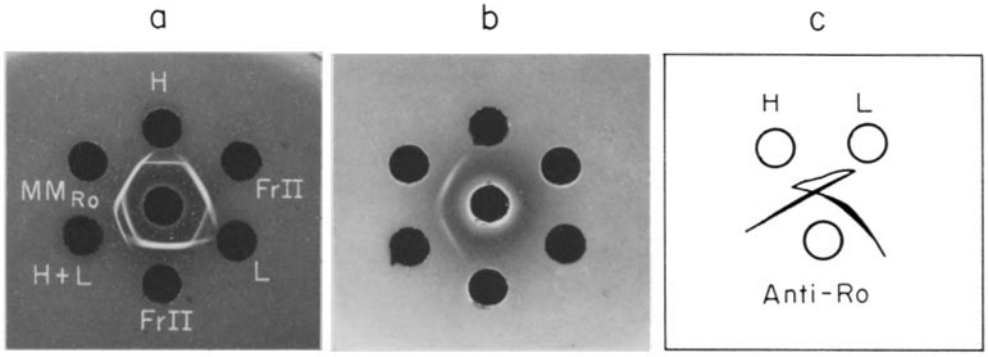

FIG. 5. Localization of individual specificity to the recombined chains of Ro protein. (*a*) The whole protein and the recombined chains (H and L) spurred over Fr II whereas the isolated chains were deficient relative to Fr II. The double band observed with the recombined chains was due to an excess of free light chains. (*b*) After absorption of the anti-Ro antiserum with 2 mg of Fr II only the recombined chains and the whole protein continued to react. (*c*) Reaction observed when the isolated Ro heavy (H) and light (L) chains were placed in adjacent wells and reacted with unabsorbed anti-Ro antiserum. The major bands gave the expected lines of non-identity; behind the intersection an irregularly positioned band was observed apparently caused by recombination of the heavy and light chains in the agar and the acquisition thereby of antigenic specificity.

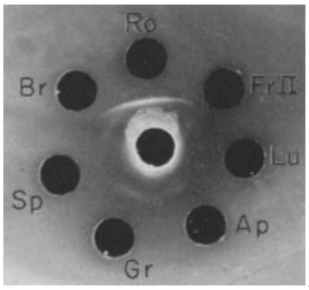

FIG. 6. Recombination of Ro heavy chains with Ro light chains and heterologous light chains. When anti-Ro antiserum absorbed with 2 mg of Fr II was reacted with the recombined chains only the homologous recombination of Ro heavy and light chains gave a good reaction of individual specificity whereas little or no reaction was obtained with the heterologous combinations.

minants on the Ro protein responsible for individual specificity were unique to the combined chains and did not merely represent a summation of the determinants present on the isolated chains. The necessity of having the recombination occur between the two homologous chains is illustrated in Fig. 6. Ro heavy chains were recombined with Ro light chains as well as with six other light chain preparations. These recombined chains were reacted with the absorbed Ro antiserum. Only the homologous combination of Ro light

and Ro heavy chains gave a strong reaction with the absorbed antiserum. Faint or no reaction was obtained with all other combinations despite other immunologic evidence that recombination had occurred with at least three of the other preparations.

Table I summarizes the data on the localization of individual specificity for all the myeloma proteins. As previously mentioned, individual specificity was uniformly localized to the Fab fragment produced by papain digestion. Three of five type L myeloma proteins contained individual specificity on the

TABLE I

Localization of Individual Specificity to γ-Globulin Subunits

Myeloma protein and chain classification	Localization of specificity			
	Papain fragments	Light	Heavy	Recombined chains
Ro (K, Ge)*	Fab	0	0	+
Le (K, We)	Fab	0	‡	+
Sz (L, We)	—	+	0	0
Sp (L, We)	—	+	0	0
Ge (K, Ge)	Fab	0	+	+
Vi (L, Vi)	Fab	0	+	0
Ke (K, We)	Fab	0	+	+
Me (L, We)	Fab	+	0	0
Ne (L, Ne)	Fab	0	+	0
Gr (K, We)	—	0	‡	+
Fe (K, Vi)	Fab	0	‡	+
Ci (K, Ge)	Fab	−	−	−

* The letters in parentheses refer to the light and heavy chain subgroups respectively.

‡ Isolated heavy chains insoluble so that antigenic analysis could not be performed.

light chain, whereas 0 of 6 type K proteins showed light chain specificity. Two other type L proteins showed all specificity to be present on the heavy chains. Two type K myeloma proteins had some individual specificity on the heavy chains; however, they showed an increase in individual specificity upon recombination with light chains as evidenced by spur formation of the recombined chains over the isolated chains despite the fact that the isolated light chains themselves contained no individual specificity of their own. In the case of the Ro protein, all the individual specificity was localized to the recombined chains with none being present on either of the isolated chains. Three of the heavy chain preparations were insoluble so that no information could be obtained regarding the antigenicity of the uncombined chains.

In several instances more than one antiserum was available to the same myeloma protein as well as different bleedings from the same rabbits at differ-

ent stages of immunization. Variations were observed, but in general the localization appeared to be related to the individual myeloma protein used for immunization.

Light Chain Specificity and Bence Jones Proteins.—The Sp antiserum, one of the type L antisera which revealed the individual specificity to be localized to the light chains, was used to determine whether the individual specificity was also located on the homologous Bence Jones protein. Fig. 7 shows the reaction obtained when anti-Sp myeloma protein antiserum was reacted against Sp light chains, Sp Bence Jones protein, and two other type L Bence Jones proteins. Lines of identity were formed between Sp light chains and Bence Jones protein; these in turn spurred over the two heterologous type L Bence Jones pro-

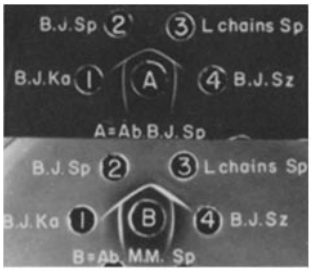

FIG. 7. Localization of individual specificity to the Bence Jones (*B.J.*) protein and light chain. A reaction of identity was formed between the Bence Jones protein and the isolated light (*L*) chains from the myeloma protein of Sp when reacted with antisera prepared against the Bence Jones protein or the myeloma protein. Both antisera were specific for the homogous protein as indicated by the spur formation of Sp light chains and Bence Jones protein over two other type L proteins, Ka and Sz.

teins. Similarly, antiserum made against Sp Bence Jones protein showed the Bence Jones protein and light chains to be identical, both showing similar individual specificity. In these and other observations on antisera made to Bence Jones proteins and isolated light chains, it became apparent that the type L proteins showed much more individual specificity than those of type K.

In several instances antisera to Bence Jones proteins gave individual specificity for the light chains of the myeloma protein which was not apparent with the antiserum to the whole myeloma protein; again, this was observed primarily with type L proteins. Similar findings were made with antisera to isolated heavy chains from myeloma proteins. Clear individual specificity for the immunizing heavy chains and the parent myeloma protein was demonstrated. In this work "chain specificity" not found in the whole protein was also encountered similar to that previously described for light chains (20) and it was necessary to distinguish such general heavy chain specificity from individual heavy chain specificity.

Observations on γA- and γM-Proteins.—Individual antigenic specificity was very apparent in studies of γA-myeloma proteins and with Waldenström macroglobulins, isolated cold agglutinins, and various 19S anti-γ-globulins. In the case of the 19S class proteins striking disappearance of the observed individual specificity was noted following reduction with mercaptoethanol with or without treatment with iodoacetamide. This was observed with most of the systems studied. However, in the two instances where detailed investigations were carried out it was found that the mercaptoethanol dissociated subunits completely inhibited the individual specific precipitation noted for the intact 19S protein. It thus appeared that the subunits still had the individual specificity despite the inability to detect it by precipitating techniques. The other macroglobulins which showed disappearance of reactivity in gel precipitation on dissociation were not investigated further to determine whether similar inhibition occurred. Two other macroglobulins showed only partial loss of individual specificity in agar gel analyses upon mercaptoethanol dissociation.

DISCUSSION

In the present study, quantitative precipitin data was obtained concerning the capacity of normal γ-globulin to absorb out antibodies specific for individual myeloma proteins. The degree of absorption was limited by the concentration of Fr II obtainable in solution and by the dilution effect inherent in such experiments. In most instances the addition of increasing amounts of Fr II continued to remove antibodies from these antisera even to the extent of complete removal in some.

The interpretation of these observations is complicated considerably by the simultaneous absorption of antibodies to the various heavy and light chain subgroups which need to be distinguished from those involving individual specificity. Certain of the heavy chain subgroups exist in less than 5 per cent of the molecules of Fr II (4) and considerable absorption is necessary to remove such antibodies. The light chain subgroups other than those involving type K and L molecules remain poorly differentiated which further complicates the problem (21–23). In one instance (Fig. 1 *c*) there was only slight absorption of antibody when 100 mg of Fr II was used per ml of antiserum. An absorption with 0.1 mg of the specific protein per ml of antiserum could be detected by the procedures used; thus, less than this quantity must have been present in the 100 mg of Fr II used for absorption. This means that if the myeloma protein represents an increase in a single species of molecule in Fr II, that species represented less than 0.1 per cent of the total γ-globulin. On the other hand, the individual specificity of other antisera could be removed to some extent by absorption with Fr II and therefore molecules containing determinants similar to those specific for the myeloma protein were present in Fr II at concentrations greater than 0.1 per cent. Although no definite con-

clusions can be drawn from these experiments the data appear most compatible with the hypothesis (8, 24) that the individual specific antigenic determinants of myeloma proteins are shared by molecules in Fr II but that the portion of Fr II that contains the particular antigenic determinants under investigation may vary and in some instances may be so small that it is difficult to influence the immunologic reactivity of a specific antiserum.

Although the capacity of normal γ-globulin to absorb antibodies to individual specificity argues against the "abnormal" nature of the antigenic structure of myeloma proteins, it is not at all clear whether there are any molecules in normal γ-globulin identical to those of the myeloma protein or whether the absorption of individual specificity is dependent on the presence of several species of γ-globulin molecules each of which share some but not all structural features with the myeloma protein. Thus in this situation, the effective absorption requires the presence of multiple species of molecules in the pooled γ-globulin. Some evidence for the latter concept was obtained with certain antisera. For example, anti-Ge antiserum after partial absorption reacted with myeloma proteins of the Ge subgroup. Most other myeloma proteins failed to react but a few were encountered in different subgroups which continued to give significant precipitation. The antigen concerned here was difficult to absorb out and could be differentiated from that involved in the subgrouping. When Fr II was studied with this antiserum in agar plate analysis, very high concentrations were required to show any reaction but a concentration of 50 mg/cc showed the development of three distinct lines against this absorbed antiserum. This and other experiments suggested that multiple components of pooled γ-globulin participated in the further absorption to diminish the individual specificity.

The localization of individual specificity uniformly to the Fab fragment produced by papain cleavage is of considerable interest since the antibody combining site of antibody molecules is located on this fragment (18). The fact that myeloma proteins are apparently antigenically unique in this portion of the molecule suggests that there is a very great variety of grossly different structural forms present in the Fab fragment of normal γ-globulin. Some information as to the variations possible within the major light chain groups is available from chemical and antigenic analysis (21–23, 25). The present report confirms these findings and extends the earlier data from this laboratory (26) with the observation that in some proteins individual specificity was localized to the light chains. This light chain localization of individual specificity was confined to type L light chains. These and other findings indicate that the capacity of type K light chains to vary is relatively limited compared to that of type L. This finding also confirms the impression obtained by peptide mapping that there is greater variation between Bence Jones proteins within type L than between type K proteins (25). Previous studies (2) as well as other

studies (27) not included in this report demonstrated that when the Bence Jones protein is used as immunizing antigen, individual specificity can be observed on group K as well as group L proteins. The present experiments also indicate that there is a similar degree of structural variation to be found in the heavy chain portion of the Fab fragment (Fd). This is of particular importance since this portion of the molecule probably contributes some of the primary sequence necessary for the antibody combining site (28–33). Although it is at present unknown whether the antigenic variation in the Fd fragment is due to differences in primary structure, the demonstration that light chains which show similar antigenic differences also differ in primary structure raises this possibility. However, it should be emphasized that the Fd fragment was not specifically isolated and that there are many difficulties with antigenic analyses of the heavy chains. Their insolubility in many instances and frequent contamination with light chain determinants which are difficult to recognize represent limiting factors in this study.

The experiments that demonstrate in certain cases heavy and light chains have to be combined in order for individual specificity to be observed points to the importance of tertiary and quaternary structure in the antigenic structure of γ-globulin. There are at least two possible mechanisms by which individual specificity might be obtained upon recombination of heavy and light chains: (*a*) Upon recombination individually specific antigenic sites on either or both chains which are hidden in the isolated chains become exposed and assume the configuration the chain has in the native molecules; (*b*) the antigenic sites responsible for individual specificity are interchain in nature in that part of a determinant is on the heavy chain and part is on a light chain so that complete determinants will only be present when the chains are combined. It is impossible at present to choose from these or other possibilities. The marked effect of dissociation with mercaptoethanol on the individual specificity of 19S proteins detected in precipitin systems was of special interest and suggests the importance of the quaternary structure in determining the antigenic specificity of these proteins as well. In at least certain instances, however, the subunits were found to still possess combining properties despite failure to precipitate.

The finding that the individual specificity of light chains is shared by the homologous Bence Jones protein extends by this very sensitive criterion the chemical and antigenic evidence (34, 35) which indicates that Bence Jones proteins in most cases are closely related to the light chains from the homologous myeloma proteins.

SUMMARY

The specific antigenic structure of individual myeloma proteins was investigated for the presence of similar antigenic determinants in pooled γ-globulin and for the localization of these determinants on the γ-globulin molecules.

Quantitative precipitin analyses demonstrated that in most instances absorption of antisera specific for an individual myeloma protein with large amounts of γ-globulin markedly reduced or completely removed the reactivity of the antiserum for the homologous myeloma protein. In only one instance did strong specificity remain after absorption with 100 mg of Fr II per cc of antiserum.

The antigenic determinants responsible for the individual specificity were localized in all cases studied solely to the Fab fragment produced by papain digestion. After reductive cleavage, three patterns of localization were observed. Individual specificity could be localized either to; (*a*) isolated heavy chains, (*b*) isolated light chains, (*c*) antigenic determinants present only when light and heavy chains were recombined.

After immunization with whole myeloma proteins, individual specificity was localized in part at least to the isolated heavy chain in four of six proteins studied. It was localized to the light chains in three of five type L proteins but in none of six type K proteins. In the instances where individual specificity of the myeloma protein was present on the light chains, it was shown that the Bence Jones protein from the same patient also contained the individual specificity. Immunization with isolated heavy or light chains furnished further evidence for the individual specificity of both types of chains.

These studies on myeloma proteins furnished evidence concerning the portions of the γ-globulin molecule subject to individual antigenic variation. The light chains, particularly the L type and the Fd portion of the heavy chains were primarily involved. Evidence for the importance of the quaternary structure was also obtained from the necessity in some instances for light and heavy chains to be associated in order for individual specificity to be observed. The Fc fragment of the heavy chains on the other hand showed very limited variation which was related to subgroup specificity.

The authors wish to thank Dr. W. Yount and Dr. J. Durant for supplying some of the serum and urine samples used in this study. The excellent technical assistance of Mrs. Melanie Barron Fisher and Miss Ruth Brooks is also gratefully acknowledged.

BIBLIOGRAPHY

1. Fahey, J. L., Heterogeneity of γ-globulins, *Advances Immunol.*, 1962, **2,** 42.
2. Mannik, M., and Kunkel, H. G., Classification of myeloma proteins, Bence Jones proteins, and macroglobulins into two groups on the basis of common antigenic characters, *J. Exp. Med.*, 1962, **116,** 859.
3. Fahey, J. L., 2 types of 6.6S γ-globulins, β_2A globulins, and 18S γ-macroglobulins in normal serum and γ-microglobulins in normal urine, *J. Immunol.*, 1963, **91,** 438.
4. Grey, H. M., and Kunkel, H. G., H chain subgroups of myeloma proteins and normal 7S γ-globulin, *J. Exp. Med.*, 1964, **120,** 253.
5. Terry, W. D., and Fahey, J. L., Subclasses of human γ_2-globulin based on differences in the heavy polypeptide chains, *Science*, 1964, **146,** 400.

6. Ballieux, R. E., Bernier, G. M., Tominaga, K., and Putnam, F. W., Gamma globulin antigenic types defined by heavy chain determinants, *Science*, 1964, **145**, 168.
7. Slater, R. J., Ward, S. M., and Kunkel, H. G., Immunologic relationships among the myeloma proteins, *J. Exp. Med.*, 1955, **101**, 85.
8. Deutsch, H. F., Morton, J. I., and Kratochvil, C. H., Antigenic identity of hyperglobulinemic serum components with proteins of normal serum. *J. Biol. Chem.*, 1956, **222**, 39.
9. Korngold, L., Antigenic specificity of γ_2 myeloma globulins, *J. Nat. Cancer Inst.*, 1963, **30**, 553.
10. Kunkel, H. G., Mannik, M., and Williams, R. C., Individual antigenic specificity of isolated antibodies, *Science*, 1963, **140**, 1218.
11. Oudin, J., and Michel, M., Une nouvelle forme d'allotypie des globulines γ du serum de lapin apparemment liee a la fonction et a la specificite anticorps, *Compt. rend. Acad. Sc.*, 1963, **257**, 805.
12. Kunkel, H. G., Zone electrophoresis, *Methods Biochem. Anal.*, 1954, **1**, 141.
13. Ouchterlony, O., Antigen-antibody reactions in gel IV, Types of reaction in coordinated systems of diffusion, *Acta Path. et Microbiol. Scand.*, 1953, **32**, 231.
14. Scheidegger, J. J., Une micro-methode d'immunoelectrophorese, *Internat. Arch. Allergy and Appl. Immunol.*, 1955, **7**, 103.
15. Kabat, E. A., and Mayer, M. M., Experimental Immunochemistry, Springfield, Illinois, Charles C. Thomas, 2nd edition, 1961.
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., Protein measurement with the Folin-phenol reagent, *J. Biol. Chem.*, 1951, **193**, 265.
17. Fleischman, J. B., Pain, R. H., and Porter, R. R., Reduction of γ-globulins, *Arch. Biochem. and Biophysics*, 1962, suppl. 1, 174.
18. Porter, R. R., The hydrolysis of rabbit γ-globulin and antibodies with crystalline papain, *Biochem. J.*, 1959, **73**, 119.
19. Grey, H. M., and Kunkel, H. G., unpublished observations.
20. Nachman, R. L., and Engle, R. L., Gamma globulin: unmasking of hidden antigenic sites on light chains, *Science*, 1964, **145**, 167.
21. Stein, S., Nachman, R. L., and Engle, R. L., Individual and subgroup antigenic specificity of Bence Jones protein, *Nature*, 1963, **200**, 1180.
22. Williams, R. C., Jr., Heterogeneity of L-chain sites on Bence Jones proteins reacting with anti-γ-globulin factors, *Proc. Nat. Acad. Sc.*, 1964, **52**, 60.
23. Epstein, W. V., and Gross, D., Naturally occurring human antibody reacting with Bence Jones proteins., *J. Exp. Med.*, 1964, **120**, 733.
24. Meshaka, G., Hurez, D., and Seligmann, M., Etudes Immunologiques sur les globulines myelomateuses de type γ, 10th Congress of the International Society of Hematology, 1964, D: 21 abstracte.
25. Putnam, F. W., Migita, S., and Easley, C. W., Structural and immunochemical relationships among Bence Jones proteins, *Protides Biol. Fluids, Proc. Colloq.*, 1962, 1.
26. Mannik, M., and Kunkel, H. G., Antigenic specificity of individual "Monoclonal" γ-globulins, *Fed. Proc.*, 1963, **22**(2), 264.
27. Solomon, A. and Kunkel, H. G., data to be published.

28. Fleischman, J. B., Porter, R. R., and Press, E. M., The arrangement of the peptide chains in γ-globulin, *Biochem. J.*, 1963, **88,** 220.
29. Utsumi, S., and Karush, F., The subunits of purified rabbit antibody, *Biochemistry*, 1964, **3,** 1329.
30. Franek, F., Nezlin, R. S., and Skvaril, F., Antibody combining capacity of different peptide chains isolated from digested and purified horse diphtheria antitoxin, *Folia microbiol.*, 1963, **8,** 128.
31. Metzger, H., and Singer, S. J., Binding capacity of reductively fragmented antibodies to the 2,4-dinitrophenyl group, *Science*, 1963, **142,** 674.
32. Roholt, O. A., Onoue, K., and Pressman, D., Specific combination of H and L chains of rabbit gamma-globulins, *Proc. Nat. Acad. Sc.*, 1964, **51,** 173.
33. Edelman, G. M., Olins, D. E., Gally, J. A., and Zinder, W. D., Reconstitution of immunologic activity by interaction of polypeptide chains of antibodies, *Proc. Nat. Acad. Sc.*, 1963, **50,** 753.
34. Edelman, G. M., and Gally, J. A., The nature of Bence Jones proteins. Chemical similarities to polypeptide chains of myeloma globulins and normal γ-globulins, *J. Exp. Med.*, 1962, **116,** 207.
35. Schwartz, J. H., and Edelman, G. M., Comparisons of Bence Jones proteins and L polypeptide chains of myeloma globulins after hydrolysis with trypsin, *J. Exp. Med.*, 1963, **118,** 41.