CLASSIFICATION OF MYELOMA PROTEINS, BENCE JONES PROTEINS, AND MACROGLOBULINS INTO TWO GROUPS ON THE BASIS OF COMMON ANTIGENIC CHARACTERS*

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(Received for publication, August 29, 1962)

In multiple myeloma plasma cells undergo uncontrolled proliferation leading to production of a protein characteristic of each line of cells. As a result, a large amount of a homogeneous protein is elaborated into the serum, and small protein molecules may be excreted in the urine as Bence Jones proteins. Several observations suggest that the multiple myeloma proteins represent marked elevation of individual moieties of normal gamma globulins (1, 2). Interest in these proteins has intensified considerably as a means of approaching various problems of normal gamma globulin and antibodies.

Previous studies have demonstrated that gamma type multiple myeloma proteins have variable cross-reactivity with antisera to normal human gamma globulin (3). The same study also provided evidence that the beta type multiple myeloma proteins cross-react with the same antisera, but to a much lesser degree. Subsequently, multiple myeloma proteins have been classified into groups 1, 2, and 3 on the basis of their cross-reactivity with antisera to normal human gamma globulin (4). The groups 1 and 2 are gamma type myeloma proteins, whereby each group has antigenic determinants specific to the group. Group 3 in this system of classification consists of β_{2A} type multiple myeloma proteins. The same investigators classified Bence Jones proteins into two groups, A and B, on the basis of their reactivity with antisera to normal human gamma globulin (5).

It has been demonstrated that the normal 7S γ-globulin cross-reacts with 19S γ-globulin, pathological macroglobulins, β_{2A} myeloma proteins, and Bence Jones proteins and that the cross-reacting antigenic determinants of 7S γ-globulin are located on the electrophoretically slow migrating fragments (subsequently called the S-fragments) of papain-digested γ-globulin (6). The genetically determined Gm factors are located on the electrophoretically fast moving fragments of papain-digested γ-globulin (subsequently called the F-fragments); whereas the Inv factors are located on the S-fragment of the 7S γ-globulin and also on the β_{2A} myeloma proteins, Bence Jones proteins, and Waldenström type macroglobulins (7, 8). Furthermore, recent studies by Edelman and collaborators (9, 10) show that the light chains from multiple myeloma proteins, cleaved from the myeloma protein molecules by mercaptoethanol

* Aided by a grant from the National Foundation.

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in presence of urea, have precisely the same electrophoretic mobility on starch gel as the Bence Jones proteins from the same patients. All these studies indicate that certain subunits of 7S γ-globulin molecules are also present in other immune globulins; *i.e.*, the macroglobulins, β_{2A} proteins, and Bence Jones proteins.

In the current investigation it was observed that the antigenic determinants which allow the classification of gamma type multiple myeloma proteins into two groups also exist on the smaller Bence Jones proteins, as well as on β_{2A} myeloma proteins and on Waldenström type macroglobulins. As a result, the analysis of these determinants permits the classification of all "immunoglobulins" into two major antigenic groups. In addition, evidence was obtained for a specific antigenic relationship between the serum myeloma protein and the Bence Jones protein excreted by the same individual.

Materials and Methods

Multiple Myeloma Proteins.—Sera were collected from patients with established diagnosis of multiple myeloma and stored at 4°C. The multiple myeloma proteins were isolated from other serum components by zone electrophoresis on starch medium (11).

All multiple myeloma proteins were classified as gamma type or beta 2A type myeloma proteins by immunoelectrophoresis utilizing specific antisera to 7S γ-globulin and to β_{2A}-globulin (12).

Bence Jones Proteins.—24-hour urine specimens were collected from patients with the diagnosis of multiple myeloma. The heat test for Bence Jones proteins was performed on fresh urine (13). The entire urine specimen was filtered through No. 40 Whatman filter paper and then dialyzed against running tap water for 24 hours in Visking dialysis tubing (31/32 inch width). Subsequently the dialyzed urine was concentrated by pervaporization at 4°C through the dialysis membrane. Zone electrophoresis on starch medium (11) was utilized to obtain further purification of Bence Jones proteins.

The specimens with negative heat test for Bence Jones proteins on native urine were concentrated as described above. Subsequently small molecular weight proteins were isolated from these concentrated specimens by sucrose density gradient ultracentrifugation (14) and then utilized for immunological studies.

Macroglobulins.—Serum was obtained from patients with Waldenström type macroglobulinemia and stored at 4°C. The diagnosis of macroglobulinemia was established either by the presence of 19S or heavier serum protein components in excess of 15 per cent of the total protein or by immunoelectrophoresis utilizing specific antisera to macroglobulins (12).

Two preparative steps were employed to obtain pure preparations of macroglobulins. Initially euglobulin was precipitated by diluting the serum with cold distilled water or cold phosphate buffer (pH 5.8; 0.2 M Na_2HPO_4; 0.1 M citric acid) in 1:15 ratio. The precipitated proteins were dissolved in 0.85 per cent sodium chloride solution. Some macroglobulins were initially isolated by zone electrophoresis on starch medium (11). Subsequently all preparations were subjected to density gradient ultracentrifugation in Spinco L centrifuge (14). Only the bottom fractions with macroglobulins were utilized for immunological studies.

Antisera.—Antisera to 7S γ-globulin were produced by hyperimmunizing rabbits with pooled human fraction II and complete Freund's adjuvant by subcutaneous weekly injections of 2 to 3 mg of fraction II over a 4 month period. Antisera to Bence Jones proteins were produced in similar manner by injecting rabbits subcutaneously with the electrophoretically isolated proteins and complete Freund's adjuvant over a 10 week course of weekly injection.

Antisera specific to β_{2A}-globulins were produced by immunizing rabbits simultaneously

with two β_{2A} myeloma proteins of different antigenic groups and subsequently absorbing these antisera with the serum of a person with congenital absence of β_{2A}-globulins (15). Antisera specific to macroglobulins were produced by immunizing rabbits with a Waldenström type macroglobulin and subsequently absorbing these antisera with 7S normal human γ-globulin.

Agar Diffusion.—Ouchterlony agar diffusion studies (16) were performed in 2.0 per cent agar (agar agar from Baltimore Biological Laboratory, Baltimore) in barbital buffer pH 8.6, ionic strength 0.05. Each antigen well received 0.25 mg of protein, and the antibody wells were filled with the antisera as indicated. At least 72 hours of incubation at 37°C was required prior to the final interpretation of the precipitin lines.

Immunoelectrophoresis was carried out as previously described (17).

Precipitin Curves.—For construction of precipitin curves the desired aliquot of antigen solution was brought to a volume of 0.2 ml with saline, and 0.1 ml of antiserum was added. The mixture was left at room temperature for 1 hour, and then stored overnight at 4°C. On the following day the precipitate was separated by centrifugation at 3000 RPM at 4°C. The precipitate was washed three times with cold saline and then dissolved in 0.1 N NaOH. The protein was determined by the Folin-Ciocalteu method (18).

The results reported in this study were obtained with pooled antiserum (No. 528) to 7S normal human γ-globulin from a single rabbit, a very potent antiserum with broad reactivity with different antigenic determinants on the γ-globulin molecule. However, similar results were obtained with other antisera in a sufficient number of experiments to demonstrate that the findings were not peculiar to antiserum No. 528. In the precipitin studies the complement in antisera was inactivated by heating to 56°C for 30 minutes.

RESULTS

Studies on Gamma Type Multiple Myeloma Proteins.—Serum and urine specimens were investigated in twenty-one patients with multiple myeloma. Fourteen of these cases had gamma type myeloma proteins (in figure legends and tables called MM proteins) in the serum as determined by immunoelectrophoresis. In four instances Bence Jones proteins (in figure legends and tables called BJ proteins) were present in urine without a myeloma protein in the serum. In three cases the serum protein peak was found to be a beta 2A type myeloma protein as revealed by immunoelectrophoresis; the latter three will be discussed under a separate heading.

Multiple myeloma proteins of the gamma type were readily classified into groups on the basis of their reactivity with antisera to normal human 7S γ-globulin, as has been described previously (4). With such antisera in agar diffusion studies the normal human γ-globulin spurred over group 1 and group 2 myeloma proteins, with a small spur over group 1 proteins and with a large spur over group 2 proteins. Representative proteins of the two groups spurred over each other, thus indicating different antigenic determinants on the proteins of each group and the presence of both determinants on normal human γ-globulin in variable proportions. These observations are illustrated in Fig. 1. On the basis of such studies the gamma type myeloma proteins were divided into group 1 and group 2; ten in the former and five in the latter group (see Table I).

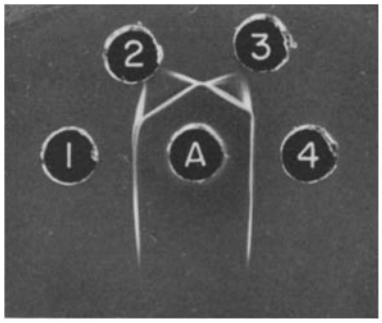

FIG. 1. Ouchterlony plate illustrating the antigenic difference between group 1 and group 2 gamma type multiple myeloma proteins. Central well *A* contains antiserum to 7S γ-globulin. Wells 1 and 4 contain human fraction II, well 2 a myeloma protein of group 2 (Spi.), and well 3, a myeloma protein of group 1 (Roy.).

TABLE I

Summary of Analyses Performed on Gamma Type Myeloma Proteins and Bence Jones Proteins from the Same Individuals

Patient	Type of MM protein	Heat test on urine	Antibody ppt'd. at equivalence	Group of MM protein	Group of BJ protein	Reaction between BJ protein and antiserum absorbed with autologous MM protein
			μg			
Cia.	γ-type	Neg.	640	1	1	0
Ger.	"	"	590	1	1	0
Haa.	"	"	660	1	1	0
Haw.	"	Pos.	590	1	1	0
Pat.	"	"	600	1	2	+
Roy.	"	"	530	1	1	0
Sel.	"	"	620	1	1	0
Sol.	"	Neg.	—	1	1	—
Tan.	"	Pos.	550	1	1	0
Wel.	"	Neg.	640	1	1	0
			Mean 602 μg SD 40.5			
Fri.	"	Pos.	380	2	2	0
Kan.	"	"	—	2	2	—
Nie.	"	Not avail.	400	2	Not avail.	Not avail.
Spi.	"	Pos.	400	2	2	0
Szo.	"	"	420	2	2	0
			Mean 400 μg SD 18.9			
Cu.	Absent	Pos.	—	—	1	—
DeM.	"	"	—	—	1	—
Fle.	"	"	—	—	2	—
Pro.	"	"	—	—	2	—

Antigenic dissimilarity between group 1 and group 2 myeloma proteins was demonstrable further by quantitative precipitin curves. Typical precipitin curves with pooled 7S human γ-globulin and representatives of the two groups of myeloma proteins are depicted in Fig. 2. The pooled fraction II gave the highest number of micrograms of protein precipitated at equivalence. The representative of group 1 myeloma proteins gave an intermediate value, and the representative of group 2 myeloma proteins precipitated the least amount of protein. The micrograms of antibody protein precipitated by various myeloma

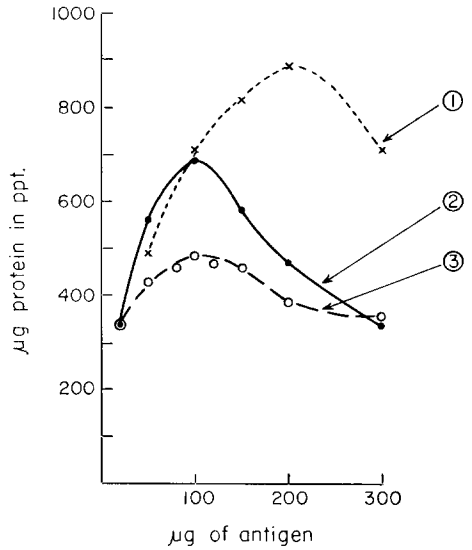

FIG. 2. Precipitin curves demonstrating the antigenic difference between groups 1 and 2 gamma type myeloma proteins. Anti-7S human γ-globulin was reacted with pooled human fraction II (curve 1), with a myeloma protein of group 1 (curve 2), and with a myeloma protein of group 2 (curve 3).

proteins at equivalence are recorded in Table I with their means and standard deviations.

In addition, the grouping of gamma type multiple myeloma proteins could effectively be carried out with antisera to Bence Jones proteins. The antiserum to a group 1 Bence Jones protein (DeM.) reacted only with multiple myeloma proteins belonging to group 1 and not with myeloma proteins from group 2. Similarly, the antiserum to a group 2 Bence Jones protein (Spi.) recognized myeloma proteins from group 2 and did not react with proteins from group 1, as illustrated in Fig. 3 by agar diffusion experiments. However, as seen in the lower half of Fig. 3, the antiserum to a group 1 Bence Jones protein did not react equally well with all group 1 proteins; two myeloma proteins of group 1

spur slightly over the protein of Ger., also a group 1 gamma type myeloma protein.

On the basis of previous work, it was thought that the antigenic determinants of myeloma proteins that allow the grouping of these proteins are located on the S-fragments of the papain-digested myeloma protein molecules. To test this hypothesis, an antiserum to normal human γ-globulin was absorbed at antigen excess with the F-fragments and with the S-fragments of papain-digested

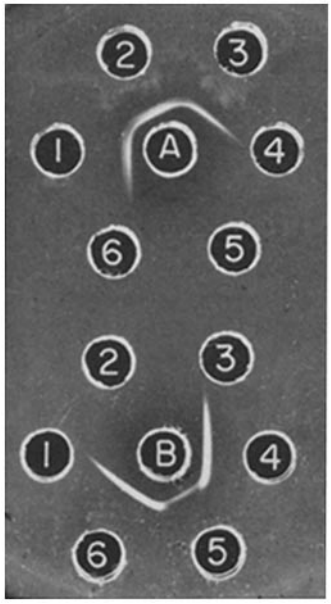

FIG. 3. Ouchterlony plate illustrating the reactivity of group 1 and group 2 gamma type myeloma proteins with antisera to Bence Jones proteins. Well *A* contains antiserum to a group 2 Bence Jones protein (Spi.), and well *B* contains antiserum to a group 1 Bence Jones protein (DeM.). Wells 1 to 3 contain group 2 myeloma proteins (Kan., Szo., Fri. respectively) and wells 4 to 6 contain group 1 myeloma proteins (Roy., Ger., Tan. respectively).

γ-globulin. The upper half of Fig. 4 shows an agar diffusion experiment with representatives of the two groups of gamma type myeloma proteins spurring over each other and the 7S γ-globulin spurring over both groups with the antiserum absorbed with F-fragments. The precipitin lines in the upper half of the figure are heavy and broad because the plate was incubated for 96 hours to allow maximal time for the development of spurs in the lower half of the plate. The spur of normal 7S γ-globulin over the group 1 protein Wel, is not well seen in the illustration. On the other hand, if the same antiserum was absorbed with the S-fragments, the same myeloma proteins and normal γ-globulin show complete identity as seen on the lower half of Fig. 4. To obtain additional support for the observation that the group specificity of myeloma proteins is located on the

S-fragments of the parent proteins, multiple myeloma proteins Spi., Ger., and Roy. were digested with papain (19). In agar diffusion experiments the group specificity was again present on the S-fragments, and the F-fragments of these proteins were identical.

Studies on Bence Jones Proteins and Their Relationship to Autologous Myeloma Proteins.—The presence of classical Bence Jones protein in the urine was ascer-

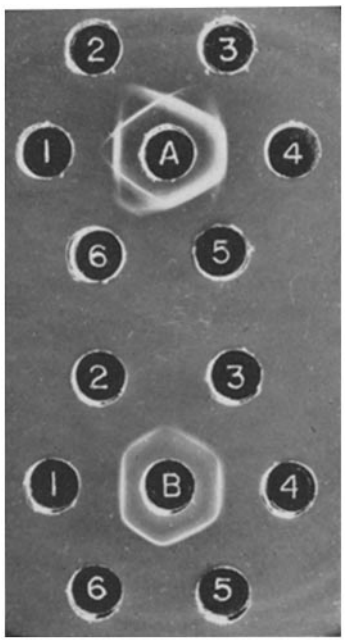

FIG. 4. Ouchterlony plate demonstrating that the antigenic specificities of multiple myeloma proteins are localized to the S-fragments. Well *A* contains anti-7S γ-globulin serum absorbed with F-fragments of normal human γ-globulin, well *B* contains the same antiserum absorbed with S-fragments of normal human γ-globulin. The upper and lower peripheral wells contain: 1, group 1 MM protein (Roy.); 2, group 2 MM protein (Spi.); 3, group 1 MM protein (Ger.); 4, fraction II; 5, group 1 MM protein (Wel.); 6, group 2 MM protein (Szo.).

tained by heating the native urine after adjusting the pH to 4.9 ± 0.1 (13). Seven of the twenty-one specimens gave a negative test for Bence Jones proteins. The classical Bence Jones proteins as well as the isolated small molecular weight proteins from urines with negative heat tests were subjected to immunological studies described below. In this section of the study, and subsequently, the two groups of Bence Jones proteins will be termed group 1 (formerly B) and group 2 (formerly A) for reasons stated below.

The antiserum to Bence Jones protein DeM. (group 1) reacted only with other Bence Jones proteins from group 1, and not with Bence Jones proteins

from group 2. On the other hand, the antiserum to Bence Jones protein Spi. (group 2) reacted only with other Bence Jones proteins from group 2 and not with group 1 proteins. These observations are illustrated in Fig. 5. In addition, the agar diffusion experiments in Fig. 5 show that the Bence Jones protein to which the antiserum has been directed spurs over other Bence Jones proteins

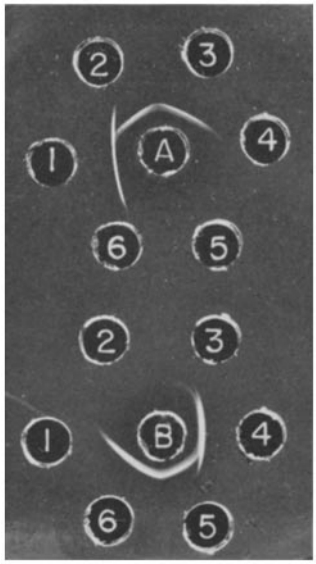

FIG. 5. Ouchterlony plate illustrating the recognition of Bence Jones proteins of group 1 (*B*) and group 2 (*A*) by antisera directed to Bence Jones proteins of these two groups. Central well *A* contains antiserum to a group 2 BJ protein (Spi.), and central well *B* contains antiserum to a group 1 BJ protein (DeM.). Peripheral wells 1 to 3 contain group 2 BJ proteins (Spi., Szo., and Fri. respectively), peripheral wells 4 to 6 contain group 1 BJ proteins (DeM., Cum., and Roy. respectively).

from the same group. This observation indicates that each Bence Jones protein has specific antigenic determinants as well as determinants in common with other members of the same group.

Previously recorded observations show that representatives of the two groups of Bence Jones proteins spur through each other when tested with antisera to 7S γ-globulin (5). Therefore, it was thought that such antisera could be utilized to group the Bence Jones proteins. An antiserum to 7S γ-globulin was absorbed at equivalence or at antigen excess with a group 2 myeloma protein. This absorbed antiserum reacted only with Bence Jones proteins from group 1 and not with proteins from group 2. Furthermore, if the same antiserum was absorbed with a myeloma protein from group 1, it reacted with Bence Jones proteins from group 2 and not with group 1 proteins. Immunoelectrophoretic experi-

ments of this type are illustrated diagrammatically in Fig. 6. The precipitin lines parallel to the antibody troughs indicate that the absorption was carried out at antigen excess.

Table I shows that nine of the patients with group 1 gamma type myeloma proteins in serum excreted group 1 Bence Jones protein in their urine. The case of Pat. formed an exception to this rule. Four patients with group 2 gamma type myeloma protein in serum excreted group 2 Bence Jones proteins in urine. Patients without a serum multiple myeloma protein excreted either group 1 or group 2 Bence Jones proteins.

Since the above observations indicated that the multiple myeloma protein and the Bence Jones protein of a patient have certain common antigenic deter-

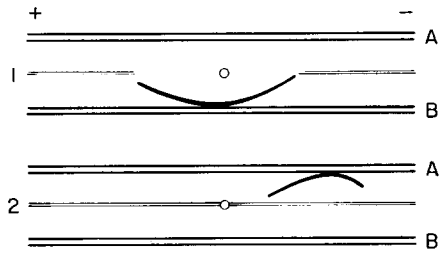

FIG. 6. Diagram of immunoelectrophoresis illustrating the recognition of Bence Jones proteins by antiserum to 7S normal γ-globulin absorbed with a MM protein of the opposite group. Trough *A*, antiserum to 7S human γ-globulin absorbed with a MM protein of group 1; trough *B*, same antiserum absorbed with a MM protein of group 2. Well 1 contains a BJ protein of group 1 (Roy.) and well 2 contains a BJ protein of group 2 (Spin.).

minants, further investigation of the relationship between these proteins was undertaken. Antiserum to normal 7S human γ-globulin was absorbed at equivalence or antigen excess with the myeloma protein of a patient. Subsequently the corresponding Bence Jones protein was tested with the absorbed antiserum. The myeloma proteins (see Table I) removed antibodies reactive with the autologous Bence Jones proteins with the exception of Pat. Diagrams of such immunoelectrophoretic studies are given in Fig. 7. The antiserum to 7S γ-globulin was also absorbed at antigen excess with the myeloma protein of Pat. As seen in Fig. 7, this absorbed antiserum still reacted with the isolated Bence Jones protein of Pat., but not with group 1 Bence Jones proteins. It is noteworthy that in this single case the Bence Jones protein group did not correspond to the serum myeloma protein group. In additional experiments antisera to individual Bence Jones proteins were absorbed at antigen excess with the myeloma protein from the same patients. After absorption these antisera failed to react with Bence Jones proteins to which they had been directed.

Studies on β_{2A} Multiple Myeloma Proteins and Their Autologous Bence Jones Proteins.—Preliminary studies with isolated beta 2A type myeloma proteins

showed that these proteins fell into two groups on the basis of their reactivity with antisera to 7S normal γ-globulin. This is illustrated in Fig. 8. The beta 2A type myeloma proteins from opposite groups spurred through each other in

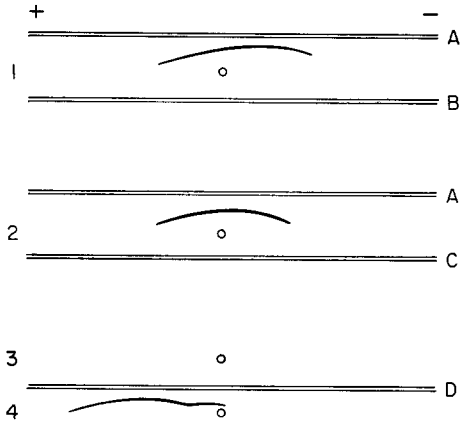

FIG. 7. Diagram of immunoelectrophoresis illustrating the antigenic similarity between the MM protein and the BJ protein from the same patient. Trough *A* contains antiserum to 7S human γ-globulin, trough *B* the same antiserum absorbed with MM protein of Roy., trough *C* the same antiserum absorbed with MM protein of Szo., and trough *D* the same antiserum absorbed with MM protein of Pat. Well 1 contains the BJ protein of Roy., well 2 the BJ protein of Szo., well 3 the MM protein of Pat., and well 4 the BJ protein of Pat.

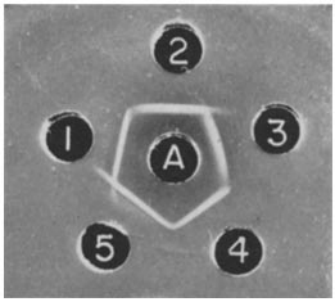

FIG. 8. Ouchterlony plate illustrating the differences between the group 1 and group 2 β_{2A} myeloma proteins. Central well *A* contains an antiserum to 7S human γ-globulin. Peripheral wells 1 and 2 contain group 2 β_{2A} MM proteins (Blo. and LuD. respectively) and wells 3, 4, and 5 contain group 1 β_{2A} MM proteins (Cot., Bem., and McC. respectively).

Ouchterlony diffusion experiments, whereas representatives of each group showed complete identity. For this reason further study was undertaken to clarify the relationship of the two groups of beta 2A type myeloma proteins to the two antigenic groups of gamma type myeloma proteins.

An antiserum to 7S normal human γ-globulin was absorbed with a group 1

gamma type myeloma protein. This absorbed antiserum reacted only with group 2 gamma type and group 2 beta 2A type myeloma proteins, showing complete identity of the group-specific antigenic determinants of the gamma and beta 2A type proteins of this group. The same antiserum was absorbed with a group 2 gamma type myeloma protein. This absorbed antiserum reacted only

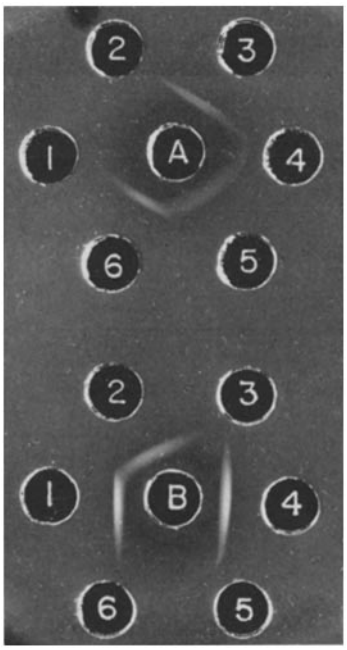

FIG. 9. Ouchterlony plate demonstrating the grouping of β_{2A} myeloma proteins. Well *A* contains an antiserum to human 7S γ-globulin absorbed with a group 1 γ type MM protein (Roy.), and well *B* contains the same antiserum absorbed with a group 2 gamma type MM protein (Spi.). Peripheral wells 1 and 4 contain a group 1 β_{2A} MM protein (Bom.), wells 3 and 6 a group 2 β_{2A} MM protein (LuD.), well 2 a group 1 gamma type MM protein (Roy.), and well 5 a group 2 gamma type MM protein (Spi.).

with group 1 gamma type and group 1 beta 2A type myeloma proteins, showing complete identity of the group-specific antigenic determinants of the gamma and beta 2A type myeloma proteins. These observations are illustrated in Fig. 9 with agar diffusion experiments. Of the eleven isolated beta 2A type myeloma proteins five belonged to group 1 and six to group 2 (Table II).

The typing of beta 2A type myeloma proteins was also performed with antisera to Bence Jones proteins. The antiserum to a group 1 Bence Jones protein (DeM.) reacted with group 1 beta 2A type myeloma proteins and not with group 2 proteins. On the other hand, an antiserum to a group 2 Bence Jones

protein (Spi.) reacted with group 2 beta 2A type proteins and not with group 1 proteins.

In three patients with beta 2A type multiple myeloma, urine specimens were available. One of these contained classical Bence Jones proteins. The three urine proteins belonged to group 2, corresponding to the group of the serum myeloma protein. The number of urine proteins investigated in beta 2A type multiple myeloma cases is small, but the same relationship appears to hold true as was observed in patients with gamma type multiple myeloma; *i.e.*, the anti-

TABLE II

Grouping of β_{2A} Myeloma Proteins and Macroglobulins by Two Different Procedures

β_{2A} myeloma proteins			Macroglobulins		
Patient	Grouping based on reaction with antisera to two types of BJ proteins	Grouping based on reaction with anti-sera to normal γ-globulin	Patient	Grouping based on reaction with antisera to two types of BJ proteins	Grouping based on reaction with antisera to normal γ-globulin
Bom.	1	1	Bem.	1	1
Cot.	1	1	Bot.	1	1
Gou.	1	1	Car.	1	1
McC.	1	1	Deu.	1	1
Xis.	1	1	Rye.	1	1
Blo.	2	2	Wol.	1	1
Cou.*	2	2	Zam.	1	1
Eis.	2	2	Fog.	2	2
LuD.	2	2	Gin.	2	2
Sam.*	2	2	Lic.	2	2
Zah.*	2	2	Lyn.	2	2
			Ran.	2	2
			Ste.	2	2

* The urine from Cou., Sam., and Zah. contained group 2 Bence Jones proteins.

genic group of the serum multiple myeloma protein corresponds to the antigenic group of the Bence Jones protein in individuals.

Studies on Waldenström type macroglobulins.—Since common group-specific antigenic determinants were observed in this study in gamma and beta 2A type myeloma proteins and in Bence Jones proteins, it was thought that these determinants should also be present on the Waldenström type macroglobulins, particularly in that the latter proteins cross-react with the S-fragments of 7S gamma globulin (6).

Indeed, the Waldenström type macroglobulins could be categorized into two groups by their reactivity with antisera to Bence Jones proteins. Antiserum to a group 1 Bence Jones protein (DeM.) reacted with only a group of isolated macroglobulins, subsequently termed group 1. This antiserum did not react

with other macroglobulins. On the other hand, antiserum to a group 2 Bence Jones protein (Spi.) reacted only with those macroglobulins that did not react with the antiserum to a group 1 Bence Jones protein. The latter group of Waldenström type macroglobulins will be subsequently termed group 2. These findings are illustrated in Fig. 10 and tabulated in Table II.

Additional experiments were conducted to establish the identity of the group-

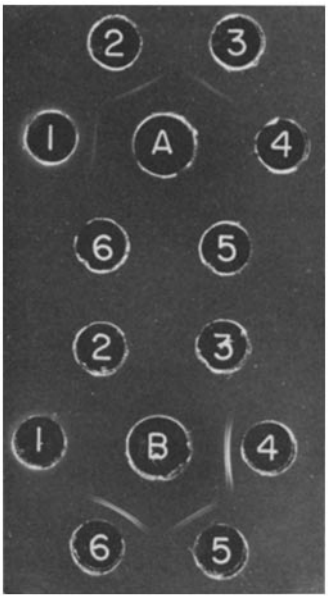

FIG. 10. Ouchterlony plate demonstrating the grouping of Waldenström type macroglobulins with antisera to Bence Jones proteins. Central well *A* contains antiserum to a group 2 Bence Jones protein (Spi.) and well *B* contains antiserum to a group 1 Bence Jones protein (DeM.). Peripheral wells 1 to 3 contain group 2 macroglobulins (Lyn., Fog., and Ste. respectively) and wells 4 to 6 contain group 1 macroglobulins (Bem., Bot., and Zam. respectively).

specific antigenic determinants on macroglobulins with the group-specific determinants on gamma type myeloma proteins. An antiserum to 7S normal γ-globulin was absorbed with the F-fragments of normal gamma globulin to avoid spur formation of the gamma type myeloma proteins over macroglobulins due to the antigenic determinants of the F-fragments of the myeloma proteins. The results of these experiments with double diffusion in agar are illustrated in Fig. 11. This illustration shows that a group 1 Waldenström type macroglobulin is identical with a group 1 gamma type myeloma protein in regard to the group-specific antigenic determinants, but reaction of non-identity is seen between a group 1 macroglobulin and a group 2 gamma type myeloma protein. Similarly a group 2 macroglobulin shows identity with a group 2 gamma type myeloma

protein and non-identity with a group 1 gamma type myeloma protein. From these observations, it is apparent that representatives of the two groups of Waldenström type macroglobulins should spur through each other with an antiserum to 7S γ-globulin. This was not observed in the studies demonstrated in the upper part of Fig. 11, possibly due to dilution of antiserum during absorption. However, using an unabsorbed potent antiserum to 7S γ-globulin,

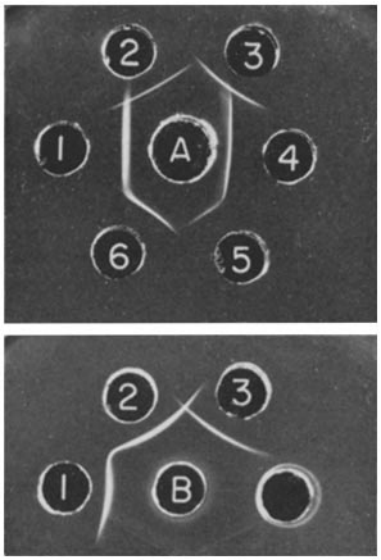

FIG. 11. Ouchterlony plate illustrating the identity of group 1 and group 2 determinants in macroglobulins and in gamma type myeloma proteins. In the upper half of this figure the central well *A* contains an antiserum to human 7S γ-globulin absorbed with F-fragments of papain-digested human fraction II. Peripheral well 1 contains a group 1 (Roy.) gamma type MM protein, wells 2 and 5 a group 2 macroglobulin (Ste.), wells 3 and 6 a group 1 macroglobulin (Bem.), and well 4 a group 2 (Spi.) gamma type MM protein. In the lower half of this figure the central well B contains an antiserum to 7S γ-globulin. Peripheral wells 1 and 2 contain group 1 macroglobulins (Bem. and Zam.) and 3 a group 2 macroglobulin (Ste.).

this could be brought out as shown in the lower half of Fig. 11. The Waldenström type macroglobulins from the two groups spurred over each other, whereas two proteins from the same group showed complete identity with this antiserum.

The cross-reaction between normal 7S γ-globulin and Waldenström type macroglobulins is not confined to the group-specific antigenic determinants described above. This was observed by absorbing an antiserum to normal 7S γ-globulin with Bence Jones proteins of both antigenic groups. Such antisera still reacted with the isolated macroglobulins, but failed to demonstrate the two groups.

Urine proteins from patients with Waldenström type macroglobulinemia were not available for analysis.

DISCUSSION

Previous observations have indicated that through the use of antisera to normal human gamma globulin the gamma type myeloma proteins could be classified into two major groups (termed 1 and 2) (4). By similar studies also Bence Jones proteins fell into two groups (termed A and B) (5). The present investigations indicate that the antigenic determinants involved in grouping of gamma type myeloma proteins and Bence Jones proteins are actually the same. Antisera to isolated Bence Jones proteins proved to be particularly useful for the classification of the two groups of gamma type myeloma proteins and the two groups of Bence Jones proteins. Equally useful for classification of these proteins were antisera to normal human gamma globulin absorbed with a myeloma protein of the opposite group. Since the antigenic determinants on gamma type myeloma proteins and Bence Jones proteins are the same, it is recommended that the group 1 and 2 nomenclature, originally used for the gamma myeloma proteins, be employed as well for the Bence Jones proteins. Further, it was demonstrated that the group-specific antigenic determinants of gamma type myeloma proteins are located on the S-fragments of the molecule. In addition, the F-fragments of myeloma proteins and normal human γ-globulin showed antigenic identity. However, the identity of the F-fragments of gamma type myeloma proteins is only antigenic. Starch gel electrophoretic studies of enzymatically degraded γ-type proteins from mouse plasma cell tumors show several distinct bands of F-fragments (20). Furthermore, the Gm genetic factors, present on the F-fragments of gamma type myeloma proteins (7), show variation among these proteins (21).

Analyses of isolated beta 2A type myeloma proteins and purified Waldenström type macroglobulins demonstrated that these, too, fell into two antigenic groups based on the presence of the determinants permitting the grouping of gamma type myeloma proteins and Bence Jones proteins. For this reason the two groups of beta 2A type myeloma proteins and the two groups of Waldenström type macroglobulins should be designated as group 1 and group 2. The grouping of these proteins was also most effectively performed by antisera to isolated Bence Jones proteins or with antisera to normal gamma globulin absorbed with a gamma myeloma protein of the opposite antigenic group. Previous studies indicated that at least two groups of beta 2A myeloma proteins existed (22, 23). Although reported investigations have demonstrated antigenic differences among the Waldenström type macroglobulins (24), the two major antigenic groups had not been described.

The present studies indicate that two fundamental antigenic types exist among gamma and beta 2A type myeloma proteins, Bence Jones proteins, and

Waldenström type macroglobulins, based on the presence of one of the two mutually exclusive antigenic determinants on these proteins. At the present time the significance of this basic difference is not apparent.

It should be emphasized that this grouping, although clear cut in most instances, represents somewhat of an oversimplification. Additional minor groups probably exist, and subgroups may become evident among these proteins with further analysis. Fig. 4 indicates that a group 1 myeloma protein (Ger.) does not react with the antiserum to a group 1 Bence Jones protein as well as the two other adjacent group 1 myeloma proteins. Similarly, occasional minor spurs were noted among the group 1 myeloma proteins in certain samples not included in this investigation. The clarification of this point requires a thorough study of a much larger series of multiple myeloma proteins and Waldenström type macroglobulins.

The present studies offer evidence that a patient with gamma type multiple myeloma protein in the serum excretes Bence Jones proteins in urine that correspond in antigenic grouping to the antigenic group of the serum myeloma protein. Furthermore, a myeloma protein contains all the antigenic determinants present on the corresponding Bence Jones protein as determined by antisera to normal human γ-globulin and with antisera to Bence Jones proteins. These results are in agreement with the recent observations of Edelman and collaborators (9, 10) on the correspondence between the Bence Jones proteins and the light chains cleaved from the myeloma proteins by mercaptoethanol in the presence of urea as revealed by starch gel electrophoresis. Several of the myeloma protein–Bence Jones protein combinations used in this study were also included in the latter investigation. Where correspondence was noted between the light chains of a myeloma protein and the Bence Jones protein on starch gel electrophoresis, also antigenic similarity was observed. The one exception reported by Edelman (10) also showed a lack of antigenic correspondence between the Bence Jones protein and the serum myeloma protein (Pat. Table I, Fig. 7). In addition, the preliminary report by Putnam *et al.* (25) on tryptic hydrolysates of myeloma proteins and the corresponding Bence Jones proteins separated on two dimensional chromatography has disclosed that the peptides of Bence Jones proteins are precisely superimposed on some of the peptides of the corresponding myeloma proteins, and that a Bence Jones protein does not contain peptides not present in the corresponding myeloma protein. One exception was also noted in their study. Thus the present immunological studies and other physicochemical investigations described above indicate that the Bence Jones proteins are closely related to the corresponding myeloma proteins and a portion of the myeloma protein behaves antigenically and physicochemically like the Bence Jones protein. The most striking evidence for this was provided by the demonstration that light chains isolated from a myeloma protein behaved like Bence Jones proteins from the same patient in being altered reversibly by heating (10).

Only a limited number of Bence Jones proteins from patients with beta 2A multiple myeloma were available. Nevertheless, grouping of the Bence Jones proteins appears to correspond to the grouping of the serum myeloma protein. Urine from patients with Waldenström type macroglobulinemia was not available for analysis. In one reported case the Bence Jones protein from such a patient was typed as group A (group 2), and from the data presented, the macroglobulin probably can be classified as from group 2 (24). It is predicted that the Bence Jones proteins from patients with Waldenström type macroglobulinemia will fall into two groups similar to Bence Jones proteins from multiple myeloma.

The observation that in double diffusion in agar normal human gamma globulin spurs over representatives of group 1 and group 2 gamma type myeloma proteins with an antiserum to normal γ-globulin suggests that the antigenic determinants allowing the grouping of myeloma proteins are also present in normal human γ-globulin, and on account of the varying intensity of spurs, perhaps in differing proportions. Indeed, studies to be reported separately in detail disclosed that approximately 60 per cent of normal γ-globulin molecules carry the antigenic determinants of group 1 and 30 per cent the antigenic determinants of group 2 myeloma proteins (26, 27). The latter observations further demonstrate the ubiquity of the two antigenic determinants among the immunoglobulins. The significance of these antigenic differences in normal 7S γ-globulin and their relationship to the production of various antibodies remain to be clarified.

SUMMARY

Antisera to normal 7S γ-globulin and to Bence Jones proteins permit the grouping of myeloma proteins (gamma and beta 2A types), Bence Jones proteins, and the Waldenström type macroglobulins into two fundamental antigenic groups. The antigenic determinants responsible for this grouping are common to all these proteins which fall in the general category of immunoglobulins. Antisera to Bence Jones proteins were particularly useful for this classification since they failed to react with the proteins of the opposite group. These antisera also permit the grouping of normal 7S γ-globulin into two major types.

The Bence Jones proteins from individual patients were found to correspond in antigenic group to that of the serum myeloma protein. Studies with antisera to 7S γ-globulin and to Bence Jones proteins indicated that the Bence Jones proteins were antigenically identical to a portion of the corresponding multiple myeloma protein molecules.

The authors wish to thank Dr. Robert W. Carey for generously providing several of the serum and urine specimens from patients with multiple myeloma.

BIBLIOGRAPHY

1. Deutsch, H. F., Kratochvil, C. H., and Reif, A. E., Immunological relation of Bence-Jones proteins to normal serum proteins, *J. Biol. Chem.*, 1955, **216**, 103.

2. Morton, J. I., and Deutsch, H. F., Some immunochemical reactions of Bence-Jones and hyperglobulinemic serum proteins of multiple myeloma patients, *Cancer Research*, 1958, **18**, 1221.
3. Slater, R. J., Ward, S. M., and Kunkel, H. G., Immunological relationships among the myeloma proteins, *J. Exp. Med.*, 1955, **101**, 85.
4. Korngold, L., and Lipari, R., Multiple-myeloma proteins. I. Immunological studies, *Cancer*, 1956, **9**, 183.
5. Korngold, L., and Lipari, R., Multiple-myeloma proteins. III. The antigenic relationship of Bence Jones proteins to normal gamma-globulin and multiple-myeloma serum proteins, *Cancer*, 1956, **9**, 262.
6. Franklin, E. C., and Stanworth, D. R., Antigenic relationships between immune globulins and certain related paraproteins in man, *J. Exp. Med.*, 1961, **114**, 521.
7. Harboe, M., Osterland, C. K., and Kunkel, H. G., Localization of two genetic factors to different areas of γ-globulin molecules, *Science*, 1962, **136**, 979.
8. Franklin, E. C., Fudenberg, H., Meltzer, M., and Stanworth, D. R., The structural basis for genetic variations of normal human γ-globulin, *Proc. Nat. Acad. Sc.*, 1962, **48**, 914.
9. Poulik, M. D., and Edelman, G. M., Comparison of reduced alkylated derivatives of myeloma globulins and Bence-Jones proteins, *Nature*, 1961, **191**, 1274.
10. 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.
11. Kunkel, H. G., Zone electrophoresis, *Methods Biochem. Anal.*, 1954, **1**, 141.
12. Heremans, J. F., and Heremans, M-Th., Immunoelectrophoresis, *Acta Med. Scand.*, 1961, suppl. **367**, 27.
13. Putnam, F. W., Easley, C. E., Lynn, L. T., Ritchie, A. E., and Phelps, R. A., The heat precipitation of Bence-Jones proteins. I. Optimum conditions, *Arch. Biochem. and Biophysics*, 1959, **83**, 115.
14. Kunkel, H. G., Macroglobulins and high molecular weight antibodies, *in* The Plasma Proteins, (F. W. Putnam, editor), New York, Academic Press, Inc., 1960, 279.
15. Rockey, J. H., and Kunkel, H. G., data to be published.
16. Ouchterlony, O., Antigen-antibody reactions in gels. IV. Types of reactions in coordinated systems of diffusion, *Acta Path. et Microbiol. Scand.*, 1953, **32**, 231.
17. Edelman, G. M., Heremans, J. F., Heremans, M-Th., and Kunkel, H. G., Immunological studies of human γ-globulin, *J. Exp. Med.*, 1960, **112**, 203.
18. 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.
19. Porter, R. R., The hydrolysis of rabbit gamma globulin and antibodies with crystalline papain, *Biochem. J.*, 1959, **73**, 119.
20. Fahey, J. L., and Askonas, B. A., Enzymatically produced subunits of proteins formed by plasma cells in mice. I. γ-globulin and γ-myeloma proteins, *J. Exp. Med.*, 1962, **115**, 623.
21. Harboe, M., Osterland, C. K., Mannik, M., and Kunkel, H. G. Genetic characters of human γ-globulin in myeloma proteins, *J. Exp. Med.*, 1962, **116**, 719.
22. Heremans, J. F., Les globulines sériques du système gamma, Brussels, Editions Arscia S. A., 1960, 192.

23. Franklin, E. C., Two types of γ_{1A} globulin in sera from normals and patients with multiple myeloma, *Nature*, 1962, **195**, 393.
24. Korngold, L., and Van Leeuwen, G., Macroglobulinemia. I. Antigenic relationship of pathological macroglobulins to normal γ-globulin, *J. Exp. Med.*, 1957, **106**, 467.
25. Putnam, F. W., Migita, S., and Easley, C. W., Comparative structural studies of abnormal globulins, *Fed. Proc.*, 1962, **21**, 77.
26. Mannik, M., Kunkel, H. G., Biro, C., and Edelman, G. M., The occurrence of antigenically deficient γ_2-globulins in normal human serum, *Fed. Proc.*, 1962, **21**, 11.
27. Mannik, M., and Kunkel, H. G., Two major types of normal 7S γ-globulin, *J. Exp. Med.*, 1963, **117**, in press.