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MYELOMA PROTEINS AND ANTIBODIES*

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I. Introduction

AGAMMAGLOBULINEMIA represents one disease which has very aptly been termed "an experiment of nature" because of the large body of widely useful information gained through clinical investigation of patients with this condition. Another such disease, at the opposite extreme with tremendous overproduction of γ-globulin, is multiple myeloma, which shows promise of proving of even greater utility and represents another area where clinical investigation has much to offer for the elucidation of a variety of basic problems in immunology.

There is no more intriguing question in biology today than that of antibody specificity. How is it acquired from the antigen and what are the permutations and combinations of the γ-globulin molecule which endows it with an individual specificity? It is a problem, however, that has proved uniquely difficult to attack, primarily because of the difficulty in isolating in workable quantities antibodies to single antigenic determinants. In fact, valid question remains whether this has ever been accomplished even in small amounts. As an alternative, the readily available myeloma proteins have been and will certainly continue to be utilized. The possibility exists that they themselves represent individual antibodies, the products of the single plasma cells that gave rise to the clone of myeloma cells. Some support for this concept has arisen recently from the discovery of antibody-like activity for a number of the Waldenström type macroglobulins (Kritzman *et al.*, 1961). Irrespective of the final settlement regarding this question, one conclusion has become apparent to all who have worked with these and the related Bence-Jones proteins and

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macroglobulins, and that is, that every piece of information gained from their study, no matter how seemingly irrelevant, is directly applicable to the problem of normal γ-globulin and individual antibodies. This point represents the main theme of this paper.

Bence Jones proteins were first described in 1845, more than a hundred years ago. The story of how Sir James Watson sent a bottle of urine to Dr. Henry Bence Jones for special study has been recounted many times in traditional British fashion. The equally and perhaps more significant discovery of the myeloma protein in the serum and its distinction from the urinary Bence Jones proteins by Geschickter and Copeland (1928) at Johns Hopkins Hospital, has scarcely been noticed. Their study initiated an interest in the relationship of the serum and urinary protein which is certainly a lively one at the present day. Mention also should be made of the early work of Dr. Stanhope Bayne-Jones on Bence Jones proteins (1922); the correspondence in names can scarcely have been fortuitious. He was one of the first to carry out immunological studies. In fact in reading through this work recently, it was rather shocking to see how similar certain of his conclusions on immunological subgroups were to some presumably original ones in a recent paper of ours. Progress in the myeloma field shiften from Baltimore to New York with the now classical studies of Gutman and associates (1941) on the physical characteristics of the serum and urinary proteins. In the more clinical area, Dr. Snapper assembled a vast amount of useful information and stimulated interest in a variety of therapeutic possibilities (Snapper *et al.,* 1953).

Our own interest in this field approximately fifteen years ago with studies employing antisera to the newly available Fr II γ-globulin (Kunkel *et al.,* 1951). This work indicated that the myeloma proteins were closely related antigenically to normal γ-globulin. The earlier investigators were more impressed with the antigenic uniqueness of the myeloma and Bence Jones proteins, a topic which is of considerable current interest and to which I will return later. Reactions with normal serum and γ-globulin had been dismissed as due to contaminants. The concept of paraproteins or abnormal proteins was firmly entrenched and even today continues to have a few adherents.

Figure 1, taken from these early studies, shows precipitin curves obtained from the reaction of an antiserum to Fr II γ-globulin and dilutions of two myeloma sera and one normal serum. The point of equivalence is shifted markedly to the left for the myeloma sera. The only explanation for these findings was that the myeloma proteins in these sera reacted with the anti-Fr II antibodies. In fact, the concentration of the myeloma

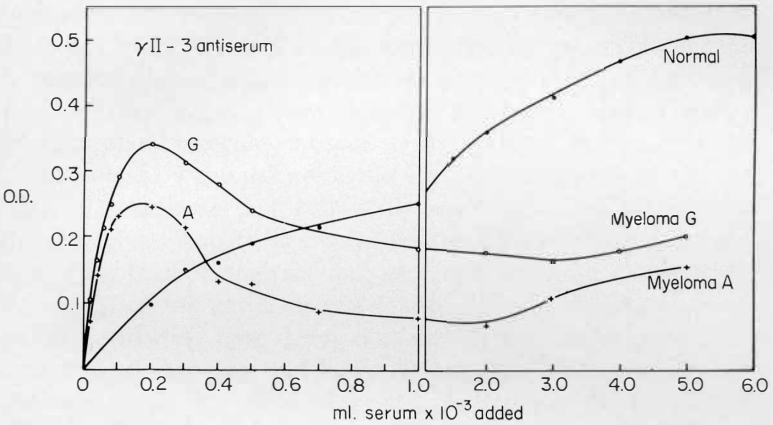

FIG. 1. Precipitin curves of serial dilutions of two myeloma sera and one normal serum with antiserum to Fr II γ-globulin indicating the reaction of antibodies to normal γ-globulin with the myeloma proteins. From Kunkel *et al.* (1951).

protein could be grossly quantitated from such curves. The lower equivalence peaks for the myeloma sera indicated that not all the antibodies to Fr II were reacting with these proteins. If one makes a mixture of myeloma proteins, making sure that group I and group II types are in the ratio found in normal γ-globulin, the resulting precipitin curve is very analogous to that of Fr II. This is also true of other properties that have been examined.

Extensive studies of this type (Slater *et al.*, 1955; Mannik and Kunkel, 1962; Mannik and Kunkel, 1963a Harboe *et al.*, 1962a) along with those of Deutsch and associates (1956), have led to the concept that γ-globulin represents a composite mixture of proteins which individually closely resemble the myeloma pro-

teins. This is illustrated semidiagrammatically for orientation purposes in Fig. 2. The broad distribution of γ-globulin in the electrophoretic spectrum of serum is illustrated along with hypothetical myeloma proteins distributed over the same mobility range as for the normal γ-globulin. Figure 2 illustrates only one dimension of difference among these proteins, that is, electrophoretic mobility. It has become evident that many others exist and that

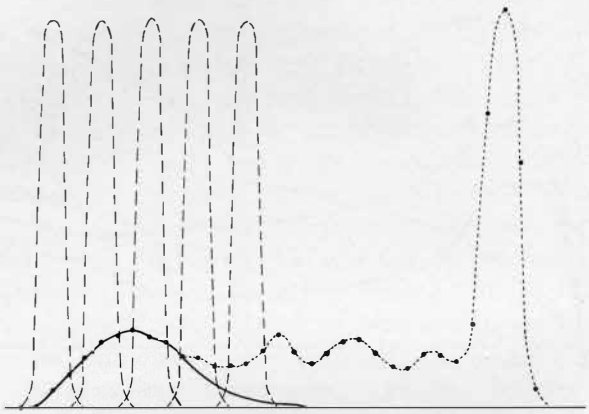

FIG. 2. Schematic drawing illustrating the broad distribution of γ-globulin in the zone electrophoresis pattern and the distribution of individual myeloma proteins over a similar area.

normal γ-globulin must be considered a myriad of different proteins each of which may have a myeloma counterpart.

II. β_{2A} Myeloma Proteins and Antibodies

Even in the earliest antigenic studies it became apparent that approximately one-fourth of the myeloma proteins behaved very differently and were very distantly related to Fr II. There was another component in normal serum which migrated between the γ and β area to which these myelomas were very similar. This finding (Slater *et al.*, 1955) led, following the development of immunoelectrophoretic techniques, to the delineation of a separate class of immunoglobulins, the β_{2A} or γ_{1A} class (Heremans, 1960; Tomasi and Zigelbaum, 1963).

In normal serum the β_{2A} class of immunoglobulins represents a minor component, of approximately one-fourth the concentration of the major 7 S type of γ-globulin. However, in other body fluids particularly external secretions, it frequently represents the dominant type of immunoglobulin (Tomasi and Zigelbaum, 1963). Current interest has centered on the assignment of antibody activity to this class and an understanding of its biological significance. A relatively simple procedure for detecting β_{2A} antibodies has been applied to the isoagglutinins (Kunkel and Rockey, 1963). Washed specific precipitates between blood group A substance and human serum containing anti-A antibodies are dissolved in antigen excess and applied to the central wall of an agar plate. Specific antisera to the various immunoglobulins in the outside wells delineate the antigenic character of the antibody. β_{2A} antisera that react only with this class of immunoglobulin give distinct lines with the dissolved antibody from most sera. Quantitative analysis indicates that in most instances less than 20% of the antibody was of the β_{2A} type. Recently, several anti-B antibodies have been obtained from human sera which show the β_{2A} type as the dominant species. Other workers have also described β_{2A} antibodies utilizing a variety of methods (Fireman *et al.*, 1963; Fahey and Goodman, 1964).

Another more complex procedure that has proved useful for the delineation of the type of antibody in specific sera has been density gradient ultracentrifugation or combination of this technique with chromatography (Rockey and Kunkel, 1962). Figure 3 illustrates the results for one anti-A serum separated first by chromatography and then by density gradient ultracentrifugation of the chromatographic fractions. Three types of antibody activity are shown which differ both in chromatographic and in sedimentation characteristics. These represent the usual 7 S and 19 S types plus an additional type with an intermediate sedimentation rate. The latter type are the subject of considerable current interest. Most of the β_{2A} antibodies in the isoagglutinin system show this property. The subject is confused, however, by the additional occurrence of isoagglutinins of intermediate sedimentation rate which are not of the β_{2A} class but appear to be polymers of ordinary 7 S γ-globulin. This type was observed in many anti-A sera.

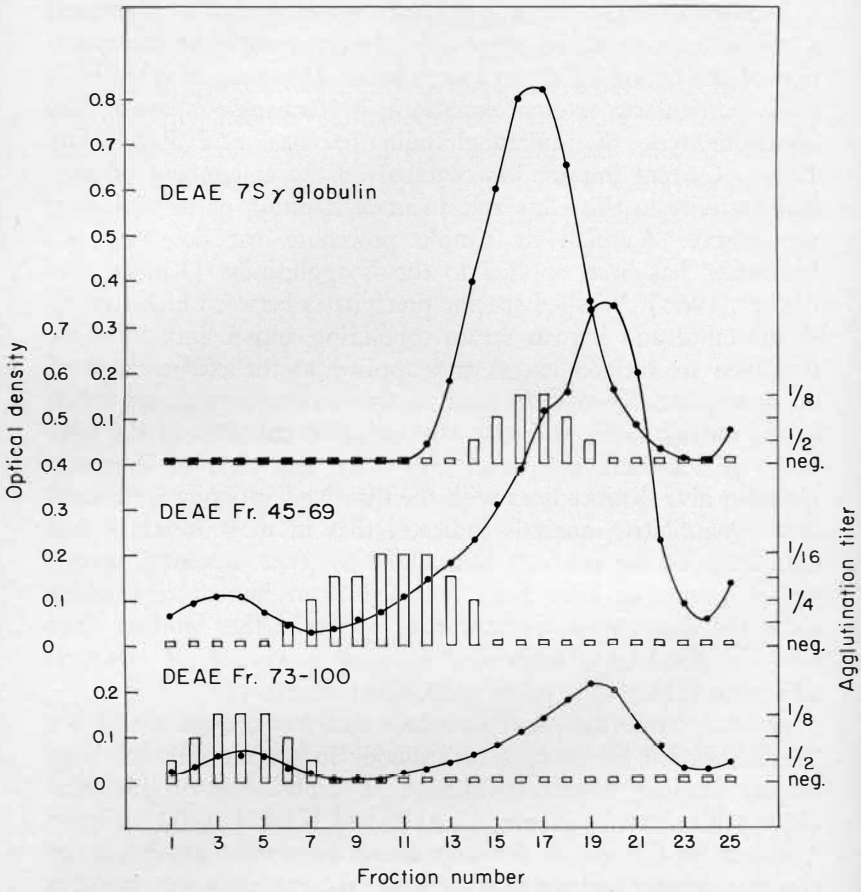

FIG. 3. Distribution of anti-A antibody activity following density gradient ultracentrifugation of three column fractions of serum. The solid lines represent the protein curves and the bars the antibody activity. The upper pattern represents 7 S antibody, the lower pattern 19 S antibody, ant the middle pattern antibody of intermediate sedimentation rate. From Rockey and Kunkel (1962).

The finding of β_{2A} antibodies of intermediate sedimentation properties was expected in view of the wide occurrence of β_{2A} type myeloma proteins which exist in polymeric forms (Laurell, 1961). As in the case of the Waldenström macroglobulins these appear to represent basic four-chain structures which are sec-

ondarily linked by readily split disulfide bonds; these polymers are very variable in size, however (Mannik and Kunkel, 1964) (cf. Fig. 4). The bulk of our knowledge concerning the β_{2A} class proteins has arisen from the study of the myeloma proteins, and in fact it is highly doubtful that we would even recognize today this important class of antibodies if it had not been for the striking properties of the myeloma proteins of this type.

III. Chain Structure of γ-Globulin and the Genetic Factors

One of the major recent developments in the γ-globulin field has been the elucidation of the multiple chain structure of these proteins. This began with observations on the dissociation of Waldenström macroglobulins with sulfhydryl compounds and has been extended to include all the immunoglobulins. It is now apparent that each of these proteins is made up of two types of polypeptide chains that differ markedly from each other. Dr. Gerald Edelman, who began this work in our laboratory, has been responsible for many of these important developments. After reduction with agents such as mercaptoethanol, the chains of all the immunoglobulins can be separated by a variety of methods. It is perhaps brought out most clearly by starch gel electrophoretic separation (Edelman and Poulik, 1961), but the chains also can be separated by a variety of other methods (Fleischman *et al.*, 1963). Here again work with myeloma proteins aided considerably, and the sharp banding of the L and H chains in gel separations were first evident with these proteins.

A dramatic finding which arose as a consequence of this work is that the Bence Jones proteins correspond to the free L polypeptide chains from the same patient (Edelman and Poulik, 1961). The recent observation (Franklin *et al.*, 1963; Osserman and Takatsuki, 1963) of patients with a myeloma-like picture who produce very large amounts of material resembling free H chains in some ways completes the picture. Both the L and H chains seem to appear in disease in the uncombined state, but thus far never in the same patient.

A primary question that arose from the elucidation of the chain picture or ordinary γ-globulin, was what relationship did

the H and L chains have to the other immunoglobulins. The answer has come from a variety of sources, but perhaps most strikingly from investigations of the genetically determined factors in γ-globulin. At least twelve different genetic types of human γ-globulin can be distinguished in different individuals. These have been studied extensively by human geneticists, and it is clear that the factors responsible are determined by genes present at a number of different loci. Two of these, the Gm and Inv loci, are clearly separate.

TABLE I

LOCALIZATION OF THE Gm AND Inv FACTORS IN THE FRAGMENTS OF γ-GLOBULIN PRODUCED BY PAPAIN

Donor	Preparation	Phenotype	
		Gm	Inv
L. B.	Whole γ-globulin	a+b−	a−b+
	F fragment	a+b−	a−b−
	S fragment	a−b−	a−b+
R. T.	Whole γ-globulin	a+b+	a+b+
	F fragment	a+b+	a−b−
	S fragment	a−b−	a+b+
L. O.	Myeloma protein	a+b−	a−b+
	F fragment	a+b−	a−b−
	S fragment	a−b−	a−b+

The Gm and Inv determinants were initially localized to different portions of the γ-globulin molecule (Harboe *et al.*, 1962b; Franklin *et al.*, 1962) through studies of the S and F fractions of γ-globulin produced by papain splitting (Table I). This work along with studies of the Inv factors in Bence Jones proteins indicated that these genetic determinants were on different polypeptide chains.

The direct demonstration of the various genetic factors on the isolated chains has been hampered considerably by technical problems involved in the isolation of the L and H chains in completely pure form. In many instances the H chains in particular are contaminated by L chains which can be detected antigenically. In addition, the H chains are in general very insoluble and are

difficult to work with. In fact the best solvent for the H chains is a solution of L chains. The strong affinity of these chains for each other even when the SH groups are blocked with iodoacetamide represents a dramatic phenomenon and makes the formation of hybrid molecules of various isolated L and H chains comparatively easy. At present there are no published data available concerning the direct localization of the GM and Inv characters to the L and H chains. Our laboratory has had limited results for a number of years which confirmed the previous experiments

TABLE II

Gm and Inv Phenotypes of the Whole γ-Globulin and L and H Chains from Different Individuals

	Native protein		L chains		H chains	
Preparation	Gm	Inv	Gm	Inv	Gm	Inv
Normal γ-globulin	a+b−x+	a−	a−b−x−	a−	a+b−x+	a−
	a+b−x+	a−	a−b−x−	a−	a+b−x+	a−
	a−b+x−	a−	a−b−x−	a−	a−b+x−	a−
	a−b+x−	a+	a−b−x−	a+	a−b+x−	a−
Myeloma protein	a+b−	a−	a−b−x−	a−	a+b−	a−
	a−b−	a−	a−b−x−	a−	a−b−	a−
	a−b−	a−	a−b−x−	a−	a−b−	a−
	a−b−	a−	a−b−x−	a−	a−b−	a−
	a−b−	a+	a−b−x−	a+	a−b−	a−

with other methods. This also has been the case with other laboratories. Recently, Dr. Mart Mannik in our group isolated the L and H chains from various preparations of normal γ-globulin and myeloma proteins, utilizing essentially the method of Fleischman *et al.*, (1963). These were used primarily for hybridization experiments, but some data on the better preparations were obtained concerning the genetic factors. Table II indicates certain of the results obtained. Unfortunately the more widely prevalent Inv(b) factor could not be measured because the reagent for this system is no longer available. However, it was clear that the Gm factors were found only on the H chains, and the Inv factors only on the L chains.

The localization of these genetic factors to the other immunoglobulins was made relatively easy through the use of myeloma proteins and Waldenström type macroglobulins. Clear-cut results were obtained which demonstrated that the Gm determinants were limited to 7 S γ-globulin whereas the Inv determinants extended to all classes of immunoglobulins including the Bence Jones proteins. Table III summarizes such results from our laboratory.

TABLE III

Gm and Inv Types of Isolated Myeloma Proteins, Macroglobulins, and Urinary Bence Jones Proteins

Class of protein	Gm type				Inv type			
	a+b−	a+b+	a−b+	a−b−	a+b−	a+b+	a−b+	a−b−
7 S γ	15	0	2	30	3	0	17	6
19 S γ	0	0	0	6	0	0	2	4
β_{2A}	0	0	0	15	1	0	9	5
Bence Jones	0	0	0	11	1	0	5	5

Experiments were also carried out on the 19 S proteins of six normal sera of different phenotypes and compared with the 7 S γ-globulin isolated from the same sera (Harboe and Osterland, 1963). This was a very laborious task and has not as yet been completed for the β_{2A} proteins (Table IV). Again, Gm activity was completely absent in the isolated 19 S material, while the Inv

TABLE IV

Genetic Types of 7 S and 19 S γ-Globulin Purified from Six Normal Sera

Serum	7 S γ-globulin		19 S γ-globulin	
1	Gm(a+b+)	Inv(a+b+)	Gm(a−b−)	Inv(a+b+)
2	Gm(a+b+)	Inv(a+b+)	Gm(a−b−)	Inv(a+b+)
3	Gm(a+b+)	Inv(a−b+)	Gm(a−b−)	Inv(a−b+)
4	Gm(a+b+)	Inv(a−b+)	Gm(a−b−)	Inv(a−b+)
5	Gm(a+b+)	Inv(a−b+)	Gm(a−b−)	Inv(a−b+)
6	Gm(a−b+)	Inv(a−b+)	Gm(a−b−)	Inv(a−b+)

type corresponded in each case to that for the 7 S fractions. In fact, the presence of Gm activity proved to serve as a useful tool in detecting contamination of 19 S fractions by 7 S γ-globulin.

Thus the L chains for each class of immunoglobulins must be very closely related and appear to be under similar genetic control. The H chains, however, are known to be completely different antigenically and in turn appear to be under independent genetic control. Recent observations on the H chain subgroups of 7 S

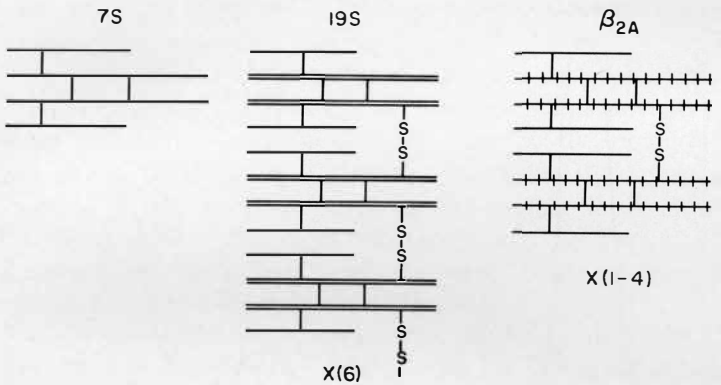

FIG. 4. Schematic diagram of the tentative chain structure of the three types of immunoglobulins. The polymeric form of the 19 S and of some β_{2A} proteins is illustrated. Each type has similar L chains and different H chains.

γ-globulin (Grey and Kunkel, 1964) indicate that each of these contain different genetic determinants controlled by genes at closely linked loci (Kunkel *et al.*, 1964). This work has led to the concept of a cluster of closely linked and partially similar structural genes in a "H chain" region of the chromosome. It seems likely that the loci involved in the H chains of 19 S and β_{2A} immunoglobulins are also closely linked to the 7 S group.

Figure 4 illustrates a tentative chain structure diagram for the three classes of immunoglobulins based on the antigenic and genetic studies mentioned above as well as starch gel electrophoresis analyses (Edelman, 1963; Carbonara and Heremans, 1963). A variety of observations indicate that the 19 S proteins

represent polymeric forms of a basic four-chain unit similar to 7 S γ-globulin. There are approximately six basic units in the 19 S class, linked by single disulfide bonds attached to the H chains (Kunkel *et al.*, 1961). The β_{2A} class is very incompletely studied at present but reduction experiments suggest a similar situation to that occurring in the 19 S class. However, in this group there is considerably greater variability in the degree of

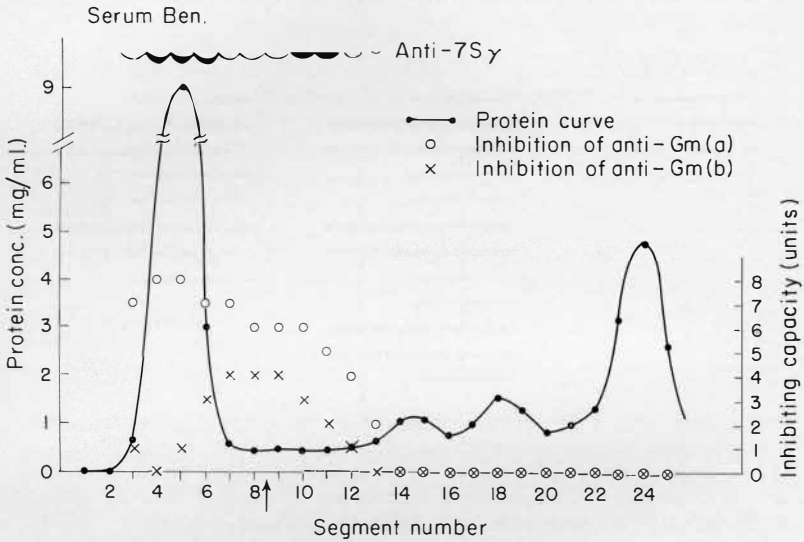

FIG. 5. Zone electrophoresis pattern of a serum containing a high concentration of myeloma protein of relatively slow mobility. The normal γ-globulin in tubes 6–12 is Gm(a + b+) whereas the myeloma protein is Gm(a + b −). From Harboe *et al.* (1962a).

polymerization ranging from a monomeric form to quite large polymers. A great heterogeneity of these forms has been observed in different myeloma proteins of the β_{2A} class.

Another finding that arose from the studies of the genetic characters in isolated myeloma proteins was the selective occurrence in these proteins of only one of each pair of apparent allelic factors in heterozygous individuals. This is illustrated in Fig. 5, showing the method of analysis for the Gm factors in both

the myeloma protein and the normal γ-globulin of that individual (Harboe *et al.*, 1962a). Each tube was brought to the same protein concentration (1 mg./ml.) for the assay. No myeloma protein typed out as Gm(a^+b^+) or Inv(a^+b^+), although Gm and Inv factors could be detected in the same myeloma. This series has been extended considerably now from work in various laboratories, and no exception to this rule has been found. This also applies when measurements are made for the new Gm(f) factor which behaves like an allele of Gm(a). Most of the myeloma proteins which were previously Gm negative are positive in the Gm(f) system.

The interpretation of these data is subject to some problems in the light of recent observations on H-chain subgroups demonstrating that Gm(b) and Gm(a) may not be the products of allelic genes (Kunkel *et al.*, 1964). However, the occurrence of Gm(f) in the same subgroup of 7 S γ-globulin as Gm(a) raises the possibility that these two factors may be controlled by true alleles, and these factors are also never found in the same myeloma proteins in heterozygous individuals.

Studies with isolated antibodies, carried out primarily by Dr. James Allen in a collaborative study with Dr. Elvin Kabat, indicate that many of these show a picture very similar to the myeloma proteins with selective occurrence of the genetic factors and frequent absence of characters which occur in the individual's γ-globulin (Allen *et al.*, 1964). The problem here is complicated by the fact that most of the antibodies studied are directed against multiple determinants. However, every indication points to the fact that the behavior of the best preparations approach closely that of the myeloma proteins.

These variable ratios of genetic factors in presumed heterozygous individuals indicate that antibody-producing cells must differ in their synthesis of proteins carrying these factors possibly reaching the extreme picture observed for the myeloma proteins. Experiments with the rabbit allotypes indicate a similar picture with variable ratios of genetic factors in different isolated antibodies (Rieder and Oudin, 1963). However, recent fluorescent antibody studies for one allelic system have not shown differential

labeling for one or the other factor in heterozygous animals (Colberg and Dray, 1964). This finding does not explain the variable ratios in different rabbit antibodies and does not fit with the findings for humans. It will be most important to resolve these apparent discrepancies.

IV. Subgroups of 7 S γ-Globulin Involving L Chains and H Chains

In addition to the genetic differences in 7 S γ-globulin multiple subclasses of 7 S γ-globulin have been found that appear to be present in all individuals. Most antisera to normal γ-globulin give a single band with Fr II γ-globulin and normal serum by immunoelectrophoretic analysis. This led many early workers, not influenced by the antigenic heterogeneity of the myeloma proteins, to consider γ-globulin antigenically homogeneous. However, many antisera clearly suggested that this was not the case. Particularly striking were antisera to β_{2A} myeloma proteins and Waldenström macroglobulins, which frequently showed double lines when allowed to react with Fr II (Edelman *et al.*, 1960). This double-line phenomenon suggested two major classes of γ-globulin. However, despite considerable effort it proved extremely difficult to demonstrate this satisfactorily. The elucidation of the phenomenon came through an appreciation of a relationship to the two classes of Bence Jones proteins that had long been known. Antisera to these Bence Jones proteins divided all classes of immunoglobulins into two groups. It had been known ever since the work of Bayne-Jones (cf. Bayne-Jones and Wilson, 1922) that at least two major antigenic groups of Bence Jones proteins existed. These were clearly brought out through the studies of Korngold and Lipari (1956). Antisera to each type were completely specific with no evidence of a cross reaction between the two. This clear difference has also become apparent through recent peptide analyses (Putnam, 1962). There are no recognizable tryptic peptides that are shared. Despite these striking differences, both types show the classical solubility properties of Bence Jones proteins.

Specific antisera to each type of Bence Jones protein divided 7 S myeloma proteins, β_{2A} myeloma proteins, and Waldenström

macroglobulins into two distinct groups termed groups I and II (Mannik and Kunkel, 1962; Fahey, 1963) (Fig. 6). Fr II γ-globulin as well as the γ-globulin from single normal individuals also consisted of a mixture of the two types which could be quantitated (Mannik and Kunkel, 1963a). Figure 7 illustrates the method employed in our laboratory utilizing I^{131}-labeled γ-globulin. Approximately 60% of the γ-globulin precipitated with antisera to group I Bence Jones proteins, and ap-

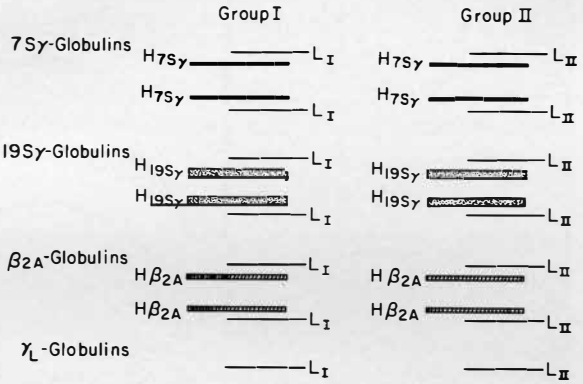

FIG. 6. Diagram of the different immunoglobulins illustrating the distribution of group I and group II L chains among all types. The γ_L refers to the free L chains found in the urine (Mannik and Kunkel, in press).

proximately 30% with the antisera to group II proteins. The combined antisera produced exact additive effects indicating that the individual molecules were not both group I and group II. As expected, isolated L chains from γ-globulin contained the two types of antigenic determinants, and these could be similarly divided by means of the Bence Jones antisera. In fact isolated L chains reacted considerably better with these antisera than did the whole γ-globulin molecules. This was due to the fact that many of the antibodies were directed against L-chain determinants that were blocked by H chains in the combined form of γ-globulin. The simple addition of H chains to L chains in recombination experiments demonstrated this phenomenon (Kunkel, Solomon,

and Grey unpublished observations). In the course of this work it was apparent that group II antisera were more heterogeneous than the group I types and contained strong specificities other than those to the group antigens.

It was of considerable interest than the 60:30 ratio of group I to group II molecules in γ-globulin was almost identical to the

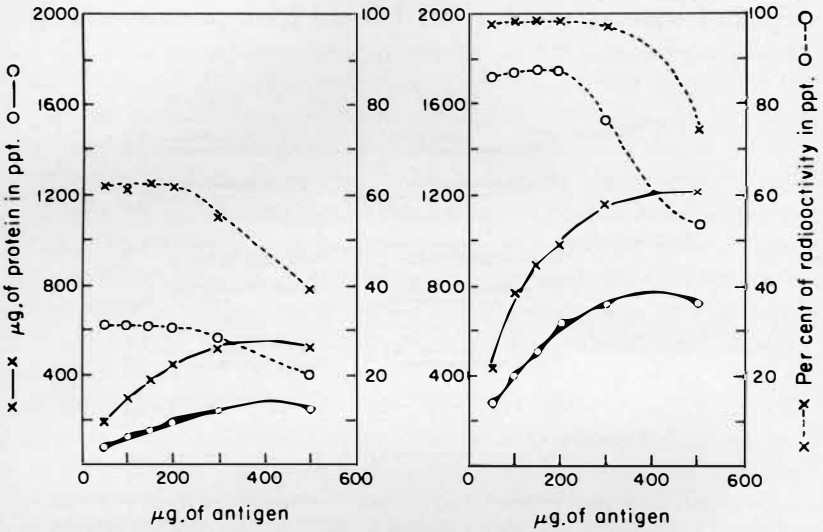

FIG. 7. Precipitin curves with I^{131}-labeled Fr II and group I and group II antisera (left), indicating the percentage of protein in each group. x——x indicates protein, and x---x indicates percentage of radioactivity precipitated by a group I antiserum; o——o and o---o indicate the same for a group II antiserum.

On the right, the curves indicate the protein and radioactivity precipitated by a mixture of group I and group II antisera (o——o, o---o) and by an antiserum to whole Fr II (x——x, x——x). From Mannik and Kunkel (1963a).

incidence of group I versus group II myeloma proteins (Mannik and Kunkel, 1963a). No evidence was obtained for a significant variation in this ratio in the γ-globulin of individuals of various ethnic and geographical groups. However, the study of isolated antibodies indicated considerable deviations in this ratio (Mannik and Kunkel, 1963b). Figure 8 illustrates the marked dif-

ference between two antiteichoic acid antibodies. Several anti-A antibodies stood out in consisting of primarily group II molecules. The cold agglutinins of seven cases of cold agglutinin disease were entirely group I. It appeared as if antibodies to single determinants might very well consist of purely one group or the other analogous to the myeloma proteins, Waldenström macroglobulins and Bence Jones proteins.

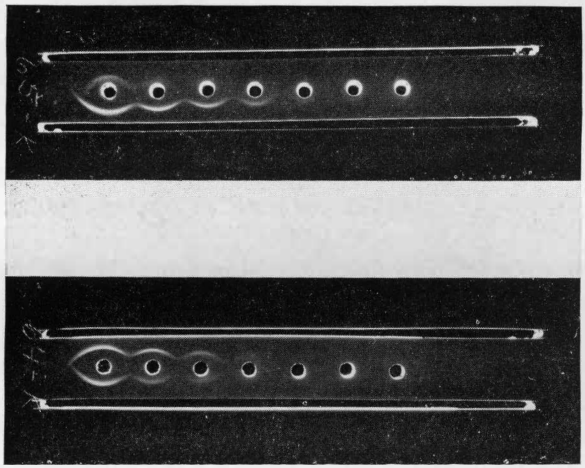

FIG. 8. Two agar slides showing the reaction of serial dilutions of two isolated antiteichoic acid antibodies with group I and group II antisera. Upper trough in each slide contains a group II antiserum, and the lower trough a group I antiserum. The upper antibody shows greatest reactivity with the group I antiserum while the lower shows a greater reactivity with the group II antiserum. From Mannik and Kunkel (1963b).

As in the case of the L chains, antigenic analyses have demonstrated a number of subgroups which reflect differences in the H chains of 7 S γ-globulin. These H-chain differences are not as complete as those for the group I and II L chains and common antigens have always been detected. Four H-chain subgroups have been delineated through the use of specific antisera to individual myeloma proteins (Grey and Kunkel, 1964). Following absorption with myeloma proteins of heterologous subgroups, specific reactions for the group concerned can be obtained. Tem-

porary names have been assigned these subgroups according to the initial myeloma protein employed as antigen in the production of the specific antisera. Quantitative precipitin studies with such antisera have permitted an estimate of the approximate quantity of the various subgroups in pooled as well as individual specimens of isolated γ-globulin. The major We group consists of approximately 60% of the total γ-globulin in Fr II, and the Vi group, approximately 8%. The Ne group and Ge group have not been as closely quantitated, but it is clear that the Ne group ranges between 10 and 20% and the Ge group represents a minor fraction. These figures agree again with the approximate incidence of myeloma proteins of each of the subgroups. A number of studies with isolated γ-globulin of representative sera from different ethnic and geographic groups have not shown any consistent difference in the relative quantity of these subgroups.

The most interesting finding arising from the study of these subgroups is that each appears to contain its own set of genetic factors (Kunkel *et al.,* 1964). In fact the two major factors in the Gm system proved to be on different group proteins. Gm(a) was found only in We group myeloma proteins, and Gm(b) only in Vi group proteins. The other two groups contained no currently recognizable Gm factors.

V. Individual Antigenic Specificity of Myeloma Proteins and Isolated Antibodies

There is one further subject which is particularly relevant with regard to the variation in the chains of γ-globulin, and this concerns the individual antigenic specificity of myeloma proteins. It has long been known that antisera to single proteins of this type react to a greater degree with the homologous antigen than with other myeloma proteins or normal γ-globulin (Slater *et al.,* 1955). This has led many to the conclusion that these are abnormal proteins. The individual specificity is brought out strikingly with many antisera by spur formation of the homologous myeloma protein over other myeloma proteins and preparations of γ-globulin. Great variation is observed from one antiserum to another in the extent of this specificity, although it is

found in the great majority of antisera. Absorption experiments with pooled γ-globulin also bring out this great variation. Some antisera lose this specificity entirely after absorption with large amounts of γ-globulin, others lose it partially, and a few are very little affected. Figure 9 shows an example of the effect of various concentrations of pooled γ-globulin on the specificity of myeloma Vi with anti-Vi antiserum. Absorption with 2 mg. of γ-globulin

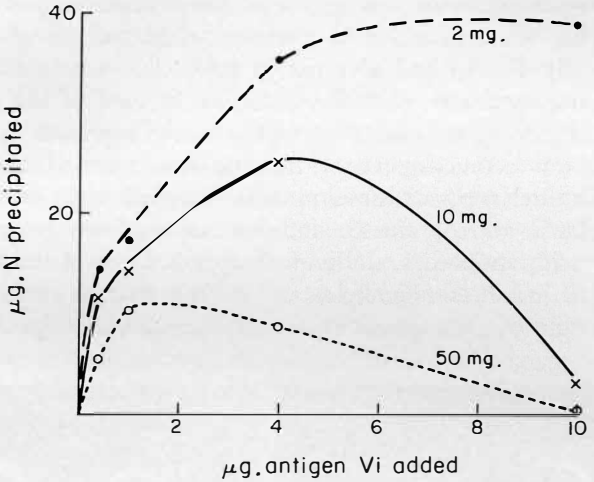

FIG. 9. Precipitin curves of isolated myeloma Vi with anti-Vi antiserum absorbed with increasing amounts of pooled γ-globulin. The amount of protein precipitated at equivalence decreases but some individual specificity remains even after the addition of 50 mg. of Fr II per milliliter of antiserum.

removes the reactivity with γ-globulin and myeloma proteins of heterologous groups. Absorption with 10 mg. removes the specificity for Vi group proteins and leaves only the individual specificity. Absorption with 50 mg. reduces this partially and 100 mg. slightly further, but some individual specificity remains. With many antisera, continued absorption with large amounts of γ-globulin gradually lowered the precipitin curve for the individual specific reaction. It appeared as if there frequently existed determinants in small amounts in pooled γ-globulin that resembled those of the antigen.

The localization of the specificity on the chains of γ-globulin has proved quite difficult. In certain instances the specificity can be demonstrated on the L chains (Mannik and Kunkel, 1963c) and in others on the H chains. However, in most instances it requires combination of the H and L chains to be evident (Grey *et al.,* to be published). The quaternary structure of the molecule appears to be most important in this type of antigenic individuality.

A large number of studies of isolated antibodies over the past twenty years have led to the conclusion that these are not antigenically distinct and give rise to antibodies closely analogous to those obtained with normal γ-globulin. In view of the striking findings regarding individual specificity in the myeloma field, this problem was reinvestigated with some new isolated antibodies against limited antigenic determinants. Animals were immunized with isolated anti-A, anti-B, anti-dextran, anti-levan, and anti-teichoic acid antibodies. Individual specificity was found in a number of instances (Kunkel *et al.,* 1963). Figure 10 illustrates one example of this specificity for an anti-A antibody. Antisera

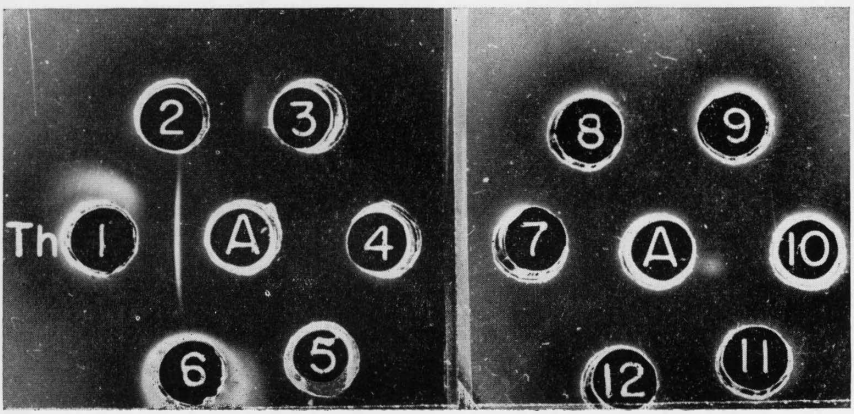

FIG. 10. Agar plate analysis showing the specific reaction of isolated antibody Th with anti-Th antiserum (A). Peripheral wells 2–5 contain γ-globulin preparations of different types, and wells 6–12 contain heterologous isolated anti-A antibodies. The antiserum (A) was absorbed with 5 mg. Fr II per milliliter of serum. From Kunkel *et al.* (1963).

to two different anti-A antibodies showed complete individual specificity and both of these failed to react in a similar fashion with any of fifteen other isolated anti-A antibodies. This has also proved true even more strikingly with the 19 S cold agglutinins. The accumulated evidence indicates that this individual specificity is unrelated to the antibody site of these antibodies.

Thus in this final distinct characteristic isolated antibodies behaved in all respects like myeloma proteins. There have been no exceptions to this rule although it is of course important to continue to be alert to this possibility.

VI. Discussion

Any discussion of antibodies would have to be considered incomplete if it did not offer some hypothesis on that question that has certainly had its share of hypotheses, the mechanism of antibody formation. Perhaps two of the points described have special relevance and offer some clues to at least the latter stages of antibody synthesis. The first is the large number of groups, subgroups, and sub-subgroups that exist for both the H and L chains with evidence of distinct chemical differences. The second concerns the commitment of the individual cell or clone of cells to a selective type of polypeptide chain. Evidence is available that group I and group II L chains can be produced by the same cell. Yet, after antibody synthesis begins, there is selective synthesis of these types. We know that the cell has the capacity to produce the allelic genetic factors in heterozygous individuals, yet the committed cell produces very variable ratios of these factors. It would seem as if the two chains most complementary to the antigen are selected for increased synthesis, probably by a de-repression mechanism, from a huge number that the cell has the capacity to make, or already produces in small amount, with continued repression of the synthesis of the remainder. The two selected chains then come together producing the final four-chain structure with even greater complementarity.

Such a concept places a dominant role on the individual cell in determining the type of protein chosen for synthesis and differs from various clonal selection theories. The multiplicity of genetic factors already discovered along with the clear indication for

many more as yet undetected, offers a tremendous source of heterogeneity for both the L and H chains. This potential is possessed by each cell and in some unknown fashion might be utilized by the cell in the production of proteins of different structures and specificities. In a process of this kind it would seem unlikely that any two individuals would ever produce exactly the same type of antibody even to the same limited antigenic determinant. The results obtained with the individual antigenic specificity of various antibodies suggest that this indeed is the case.

The time is near for a large-scale chemical attack on this problem, and the myeloma and Bence Jones proteins will undoubtedly serve as the subject materials. The antigenic approach described above, which has served to delineate subgroups involving both the L and H chains and multiple genetic factors, has thus aided in providing an overall picture of this unique and complex system and has furnished a more rational basis on which the chemistry can eventually evolve.

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REFERENCES

Allen, J. C., Kunkel, H. G., and Kabat, E. A. (1964). *J. Exptl. Med.* **119,** 453–465.

Bayne-Jones, S., and Wilson, D. W. (1922). *Bull. Johns Hopkins Hosp.* **33,** 119–125.

Carbonara, A. O., and Heremans, J. F. (1963). *Arch. Biochem. Biophys.* **102,** 137–143.

Colberg, J. E., and Dray, S. (1964). *Immunology* **7,** 273–290.

Deutsch, H. F., Morten, J. I., and Kratochvil, C. H. (1956). *J. Biol. Chem.* **222,** 39–51.

Edelman, G. M. (1963). *In* "Immunopathology, IIIrd International Symposium 1963" (P. Grabar and P. A. Miescher, eds.), pp. 57–78. Schwabe, Basel.

Edelman, G. M., and Poulik, M. D. (1961). *J. Exptl. Med.* **113,** 861–884.

Edelman, G. M., Heremans, J., Heremans, M.-Th., and Kunkel, H. G. (1960). *J. Exptl. Med.* **112,** 203–223.

Fahey, J. L. (1963). *J. Immunol.* **91,** 438–447.

Fahey, J. L., and Goodman, H. (1964). *Science* **143,** 558–590.

Fireman, P., Vannier, W. E., and Goodman, H. C. (1963). *J. Exptl. Med.* **117,** 603–619.

Fleischman, J. B., Porter, R. R., and Press, E. M. (1963). *Biochem. J.* **88,** 220–228.

Franklin, E. C., Fudenberg, H., Meltzer, M., and Stanworth, D. R. (1962). *Proc. Natl. Acad. Sci. U. S.* **48,** 914–922.

Franklin, E. C., Meltzer, M., Guggenheim, F., and Lowenstein, J. (1963). *Federation Proc.* **22,** 264.

Geschickter, C. F., and Copeland, M. M. (1928). *Arch. Surg.* **16,** 807–863.

Grey, H. M., and Kunkel (1964). *J. Exptl. Med.* **120,** 253–256.

Grey, H. M., Kunkel, H. G., and Mannik, M. *J. Exptl. Med.* (in press).

Gutman, A. B., Moore, D. H., Gutman, E. B., McClellan, V., and Kabat, E. A. (1941). *J. Clin. Invest.* **20,** 765–783.

Harboe, M., and Osterland, C. K. (1963). *In* "Immunopathology, IIIrd International Symposium 1963" (P. Grabar and P. A. Miescher, eds.), pp. 13–24, Schwabe, Basel.

Harboe, M., Osterland, C. K., Mannik, M., and Kunkel, H. G. (1962a). *J. Exptl. Med.* **116,** 719–738.

Harboe, M., Osterland, C. K., and Kunkel, H. G. (1962b). *Science* **136,** 979–980.

Heremans, J. (1960). "Les globulines sériques du système gamma." Arscia, Brussels and Paris.

Korngold, L., and Lipari, R. (1956). *Cancer* **9,** 262–272.

Kritzman, I. J., Kunkel, H. G., McCarthy, J., and Mellors, R. C. (1961). *J. Lab. Clin. Med.* **57,** 905–917.

Kunkel, H. G., and Rockey, J. H. (1963). *Proc. Soc. Exptl. Biol. Med.* **113,** 278–281.

Kunkel, H. G., Slater, R. J., and Good, R. A. (1951). *Proc. Soc. Exptl. Biol. Med.* **76,** 190–193.

Kunkel, H. G., Rockey, J. H., and Tomasi, T. (1961). *In* "Immunochemical Approaches to Problems in Microbiology" (Michael Heidelberger and Otto J. Plescia, eds.), pp. 30–42. Rutgers Univ. Press, New Brunswick, New Jersey.

Kunkel, H. G., Mannik, M., and Williams, R. C. (1963). *Science* **140,** 1218–1219.

Kunkel, H. G., Solomon, A., and Grey, H. M. Unpublished observations.

Kunkel, H. G., Allen, J. C., Grey, H. M., Mårtensson, L., and Grubb, R. (1964). *Nature* **203,** 413–414.

Laurell, A. H. F. (1961). *In* "Studies on 'Abnormal' Serum Globulins (M-components) in Myeloma, Macroglobulinemia and Related Diseases,"

pp. 69–86. Lund, Sweden. Also published as supplement 367 to *Acta Med. Scand.*

Mannik, M., and Kunkel, H. G. (1962). *J. Exptl. Med.* **116,** 859–877.

Mannik, M., and Kunkel, H. G. (1963a). *J. Exptl. Med.* **117,** 213–230.

Mannik, M., and Kunkel, H. G. (1963b). *J. Exptl. Med.* **118,** 817–826.

Mannik, M., and Kunkel, H. G. (1963c). *Federation Proc.* **22,** 264.

Mannik, M., and Kunkel, H. G. Immunological Diseases. Review. (1964) (In press.)

Osserman, E. F., and Takatsuki, K. (1963). *Medicine* **42,** 357–384.

Putnam, F. W. (1962). *Biochim. Biophys. Acta* **63,** 539–544.

Rieder, R. F., and Oudin, J. (1963). *J. Exptl. Med.* **118,** 627–633.

Rockey, J. H., and Kunkel, H. G. (1962). *Proc. Soc. Exptl. Biol. Med.* **110,** 101–105.

Slater, R. J., Ward, S. M., and Kunkel, H. G. (1955). *J. Exptl. Med.* **101,** 85–108.

Snapper, I., Turner, L. B., and Moscovitz, H. L. (1953). "Multiple Myeloma" Grune & Stratton, New York.

Tomasi, T. B., and Zigelbaum, S. (1963). *J. Clin. Invest.* **42,** 1552–1560.