GENETIC CHARACTERS OF HUMAN γ-GLOBULINS IN MYELOMA PROTEINS*,‡

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Tumors of the plasma cell series often produce large amounts of proteins which may be found in serum in the form of 7S γ-globulin, 19S γ-gloublin, β_{2A}-globulin, or Bence Jones protein, and in the urine primarily as Bence Jones protein. Although instances of heterogeneous myeloma proteins have been described, both in man (1–3) and in transplantable plasma cell tumors in mice (4–7), these proteins are usually extremely homogeneous when compared with their normal counterparts which are highly heterogeneous in a number of physicochemical properties. They lend themselves to isolation in relatively large amounts and high degree of purity, thus being well suited for physicochemical and serological studies. Although these proteins fall into four distinct classes, they are related in structure as evidenced by serological cross-reactions, and all cross-react with normal 7S γ-globulin (1, 8–13). In addition, each appears to have a counterpart in normal serum where the 7S γ-, 19S γ-, and β_{2A}-classes of immunoglobulins are now clearly recognized. Current evidence indicates that they represent three classes of antibodies (14, 15).

In recent years, a number of genetically determined factors have been described in human 7S γ-globulin. Of the seven factors clearly defined, Gm(a) (16), Gm(b) (17), Gm(x) (18), and Gm(r) (19) are determined by genes at one locus. In whites, factors Gm(a) and Gm(b) are produced by genes which behave as alternate alleles (17), whereas both factors in Negroes appear to be produced by a single allele called Gm^{ab} (20). Two alternate alleles at another locus determine the factors Inv(a) and Inv(b) (21–22). The Gm and Inv loci are independent in population studies (21, 23); the frequencies of the different Inv types make linkage studies difficult, and a slight linkage between the two loci has so far not been excluded. The seventh factor, Gm-like (24), occurs in Negroes and is rare in most other ethnic groups (25). So far, only one Gm-like(+) individual has been found in whites among thousands of individuals tested (26). The relationship between the Gm locus and the locus of Gm-like has not

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been delineated due to the fact that all Negroes are Gm(a+b+) (20, 25); the Gm-like locus is also independent of the Inv locus in population studies (25).

The primary purpose of the present study was to attempt to delineate these genetic characters in the products of plasma cell tumors particularly with the objective of discerning alterations, possibly related to the malignant process, in a comparison with the individual's normal γ-globulin. In view of the close relationship of 19S γ-globulins, β_{2A}-globulins and Bence Jones proteins to the major group of 7S γ-globulins, these proteins were also studied for the presence of the different factors. The normal genetic factors were observed in representatives of all types of myeloma proteins. Certain unusual distributions of genetic factors were observed but these were explainable by the concept that these proteins represented extreme elevations of individual moieties in the spectrum of γ-globulins rather than that the proteins were truly abnormal because of the malignant process.

TABLE I

Test System for the Gm(a) Factor Showing Inhibition by Gm(a+) Normal γ-Globulin

Reagents: Anti-Gm(a) Smejsa diluted 1:35.
Group O Rh_o red cells coated with anti-Rh_o J. J.

7S γ-globulin concentration	Gm(a+)	Gm(a−)
mg/ml		
2.0	0	3
1.0	0	3
0.50	0	3
0.25	0	3
0.12	0	3
0.06	0	3
0.03	0	3
0.015	1	3
0.008	2	3

Controls: Anti-Gm(a) + saline + coated cells: 3
Anti-Gm(a) + saline + uncoated cells: 0
Coated cells + saline: 0
7S γ-globulins + saline + coated cells: 0

Materials and Methods

Sera were obtained from patients with a clinical diagnosis of multiple myeloma or Waldenström's macroglobulinemia. In a few instances, sera were obtained from patients with inconclusive or no evidence of multiple myeloma when a sharp "monoclonal" peak appeared on agar electrophoresis. When purified, these proteins gave similar results as the proteins of typical multiple myeloma patients. They are, therefore, not discussed as a separate group.

Purification Procedures.—Myeloma proteins were purified by zone electrophoresis on starch as previously described (27). One serum (Piz. in Fig. 1) was separated, using pevikon as supporting medium (27 a). The proteins were eluted from half-inch segments, and the seg-

ment corresponding to the center of the myeloma peak used for typing. In some instances, the proteins eluted from a series of segments were brought to the same concentration and tested for inhibiting capacity in the specific test systems described later. All 7S γ-myeloma proteins referred to gave a sharp monoclonal peak on agar electrophoresis. 19S γ-globulins were purified from sera of patients with Waldenström's macroglobulinemia by procedures adapted in each case to the physicochemical properties of the macroglobulin. Bence Jones proteins were obtained by $(NH_4)_2SO_4$ precipitation or concentrating urine which had been extensively dialyzed against running tap water. Zone electrophoresis on starch was used as the final purification step in many instances. The macroglobulin and Bence Jones protein preparations were tested for purity by analytical ultracentrifugation in a Spinco model E ultracentrifuge, immunoelectrophoresis (28), and double diffusion tests in agar (12).

Classification of Myeloma Proteins.—The myeloma proteins were classified as 7S γ-globulins or β_{2A}-globulins by immunological techniques using antisera which had been adsorbed so

TABLE II

Reagents for Determination of Gm and Inv Types

Factor	Agglutinator	Anti-D	No. of dilutions tested of myeloma protein*	Inhibiting capacity‡	
				Positive 7S γ-globulin	Negative 7S γ-globulin
Gm(a)	Smejsa	J. J.	5	>5	0
Gm(b)	A. Berg	N. Berg	5	5	0
Gm(x)	Tenoux	26 R	4	>5	0
Gm-like	Carp	Warren	4	4	0
Inv(a)	Travnikova	Roehm	4	≧4	0
Inv(b)	Lucas	Ham	4	>4	0

* Starting at a concentration of 1 mg/ml of purified protein.

‡ Number of doubling dilutions which inhibit the agglutination, starting at 1 mg/ml.

they reacted specifically with each kind of protein. The 7S γ-myeloma proteins and Bence Jones proteins were classified in groups I and II according to criteria to be described elsewhere (29). The classification of myeloma proteins corresponds directly to Korngold's grouping (10). Bence Jones protein group I corresponds to group B, and group II to group A in Korngold's classification (11).

Genetic Typing.—Tests for Gm(a), Gm(b), and Gm(x) factors were made by a slide technique (30). The test system for determination of the Gm(a) factor is illustrated in Table I. Common to all reactions were group O, Rh-positive red cells coated with anti-Rh_o J.J., and a constant, optimal, dilution of anti-Gm(a) Smejsa. It is seen that Gm(a−) normal 7S γ-globulin separated by zone electrophoresis on starch was not able to inhibit the agglutination at a concentration of 2 mg/ml, whereas similarly purified Gm(a+) 7S γ-globulin inhibited the agglutination completely in seven successive doubling dilutions. Purified myeloma proteins were tested in five doubling dilutions starting at a protein concentration of 1 mg/ml. In order to regard the myeloma protein as Gm(a+) it was required that all these five dilutions inhibit the agglutination completely, whereas the presence of strong agglutination in all five dilutions made us regard the myeloma protein as Gm(a−). Only these two reaction patterns were observed. The test systems for the other factors are shown in Table II. Tests for Gm-like, Inv(a), and Inv(b) were made with a tube technique (26). All agglutinators were from non-rheumatoid sera. The reactions were read with a blind technique, and the results were easily reproducible. The term "inhibiting capacity (units)" used in the figures refers to the number

of doubling dilutions which completely inhibited the agglutination, starting at a protein concentration of 1 mg/ml. Protein determinations were performed by a modified Folin-Ciocalteu method, using Cohn Fr II γ-globulin as standard.

RESULTS

Gm Characters in 7S γ-Myeloma Proteins.—Since the inheritance of the Gm(a) and Gm(b) types is different in whites and Negroes, the findings on isolated

TABLE III

Gm and Inv Types of Isolated 7S γ-Myeloma Proteins from American Whites

Gm(a+b−)	15	Inv(a+b−)	3
Gm(a+b+)	0	Inv(a+b+)	0
Gm(a−b+)	2	Inv(a−b+)	19
Gm(a−b−)	30	Inv(a−b−)	6
Number tested.....	47		28

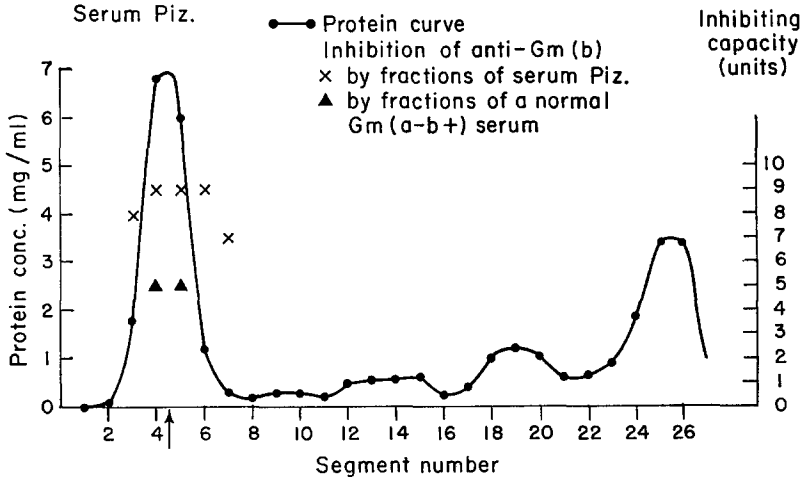

FIG. 1. Zone electrophoresis of serum Piz. showing that the myeloma protein is Gm(b+) and 16 times more inhibiting than normal Gm(a−b+) γ-globulin of similar electrophoretic mobility. The proteins of each segment were brought to the same concentration before testing. In all figures, "inhibiting capacity (units)" refers to the number of doubling dilutions which completely inhibited the agglutination of the respective test systems, starting at a concentration of 1 mg/ml.

myeloma proteins will be described separately for these two ethnic groups. The Gm(a) and Gm(b) types of forty-seven isolated 7S γ-myeloma proteins obtained from whites are included in Table III. Fifteen of them were Gm(a+b−) and two Gm(a−b+), whereas none possessed both characters.

These frequencies are strikingly different from those in normal sera of white Americans where 91 per cent are Gm(b+) and 43 per cent Gm(a+b+) (20).

The inhibiting capacity of isolated myeloma proteins was subsequently compared with that of similarly isolated 7S γ-globulin from normal individuals of the same Gm types. Of seven Gm(a+b−) myeloma proteins, three gave identical inhibition and four inhibited in one more doubling dilution than Gm(a+b−) normal γ-globulin–tested in equivalent concentrations. In contrast, two Gm(a−b+) myeloma proteins had a definitely greater inhibiting capacity than Gm(a−b+) normal γ-globulin. One of them inhibited in four and the other in three additional doubling dilutions when directly compared with normal γ-globulins of the same Gm type and identical electrophoretic mobility. One of these comparisons is illustrated in Fig. 1.

TABLE IV

Gm Types of Whole Serum and Isolated 7S γ-Myeloma Protein from Forty American Whites

Gm types of isolated 7S γ-myeloma proteins	Gm types of whole serum			
	a+b−	a+b+	a−b+	a−b−
a+b−	2	11	0	0
a+b+	0	0	0	0
a−b+	0	1	1	0
a−b−	3	3	14	5

The Gm types of the myeloma protein and the normal γ-globulin of the same sera were compared using three different approaches: first, by comparing the inhibiting capacity of whole serum with that of the isolated myeloma protein. The results are given in Table IV. In thirty cases, a number of dilutions of whole serum inhibited the agglutination in the Gm(b) test system, whereas the isolated myeloma protein typed as Gm(b+) in only two of these cases. Such findings indicate that the normal γ-globulin often is Gm(b+) when the myeloma protein is Gm(b−). Or, to express it in a different way: typing of whole serum indicated that thirty sera were Gm(b+), whereas this character was present in the myeloma protein in only two (about 7 per cent) of these individuals. Similar experiments with regard to the Gm(a) factor gave different results to the effect that the Gm(a) type of the myeloma protein was identical with that of the normal γ-globulin in thirteen (65 per cent) of twenty cases. In the remaining seven cases, the myeloma protein was Gm(a−) when the normal γ-globulin appeared to be Gm(a+). In five cases, both myeloma protein and whole serum typed as Gm(a−b−), indicating that the amount of normal γ-globulin was too low to show up in the test systems. This interpretation was borne out by the findings in agar- and immunoelectrophoresis. The finding of whole myeloma sera being Gm(a−b−) has been reported previously (30 a).

The second approach is illustrated in Fig. 2, which shows the protein curve after zone electrophoresis of myeloma serum Mar. The proteins eluted from each of 24 segments were brought to the same concentration and tested for inhibiting capacity in the Gm(a) and Gm(b) test systems. The antigenic character as determined by agar diffusion is indicated by the lines in the upper portion of the chart. No inhibition of anti-Gm(a) was observed by segments

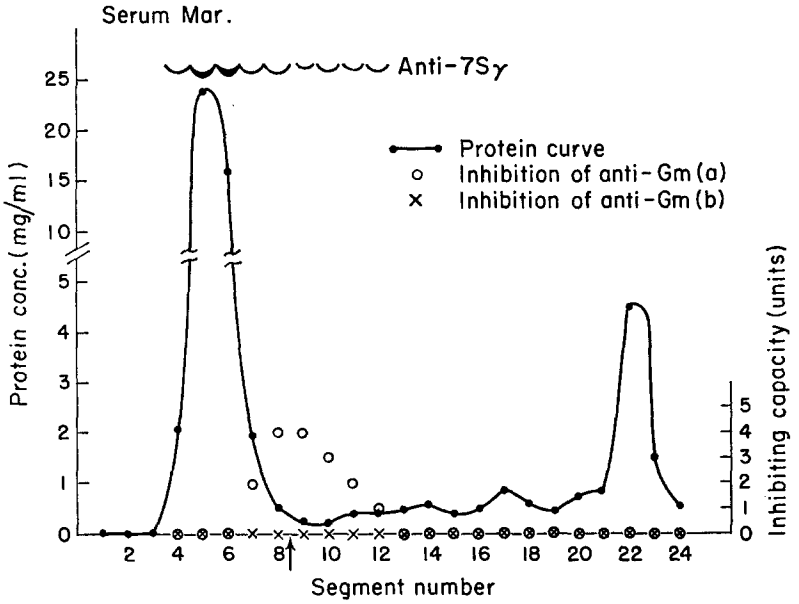

FIG. 2. Zone electrophoresis of serum Mar., showing that the myeloma protein is Gm(a−b−), whereas the normal γ-globulin ahead of the peak is Gm(a+b−). The antigenic character of the proteins of each segment was determined by agar diffusion tests using specific antisera as indicated by the lines in the upper portion of the figure.

4, 5, and 6, which corresponded to the myeloma protein. Segments just ahead of the peak (8 to 12) which contained normal γ-globulin did however inhibit anti-Gm(a). Segments 13 through 24 did not inhibit and did not contain 7S γ-globulin as judged from double diffusion tests in agar. None of the segments, nor the whole serum, inhibited anti-Gm(b). In this case, therefore, the normal γ-globulin was Gm(a+b−) and the myeloma protein Gm(a−b−). This technique was used in fifteen cases. In all instances it gave the same results as the interpretation after typing of whole serum and isolated myeloma protein. None of the cases investigated indicated that the Gm(a) character could be present in the myeloma protein when it was absent in the normal γ-globulin.

The third approach was used in serum Wei., where the myeloma protein was a 7S γ-globulin which precipitated in the cold (cryoglobulin). The serum was diluted 1:5 with distilled water. About 95 per cent of the myeloma protein was removed by subsequent precipitation in the cold. After washing, it was dissolved in saline and typed as Gm(a−b−). The supernatant, which contained the other serum proteins and a trace of myeloma protein, was dialyzed against saline and concentrated to the original serum volume. It typed as Gm(a+b+)

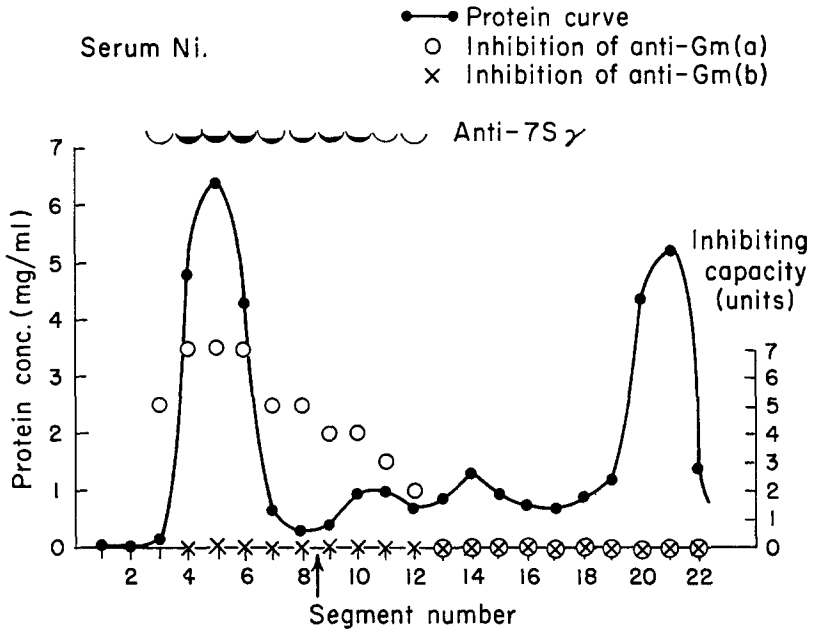

FIG. 3. Zone electrophoresis of serum Ni., showing that the myeloma protein is Gm(a+), as is the normal γ-globulin on both sides of the peak.

thus directly demonstrating that a myeloma protein may be Gm(a−b−) when the normal γ-globulin in the same serum is Gm(a+b+).

The possible effects of contaminating normal γ-globulin in the myeloma protein preparations were considered carefully. It is clear that when the peak types as Gm-negative, it may safely be regarded as Gm-negative. When there is inhibition, this may be due to the protein itself or to contaminating normal γ-globulin. Figs. 3 and 4 illustrate the experimental approach to this problem. Fig. 3 shows the zone electrophoresis of serum Ni. which typed as Gm(a+b−) in whole serum. When the proteins from individual segments of the block were tested, it was found that the normal γ-globulin on both sides of the peak inhibited anti-Gm(a), and so did the myeloma protein to a somewhat greater

extent. The comparison was made, referring to equivalent protein concentrations, and showed that the myeloma protein itself was Gm(a+). Fig. 4 shows the findings on serum Ha. which is from a Negro. The whole serum typed as Gm(a+b+). Again, the normal γ-globulin on both sides of the peak inhibited in the Gm(a) test system, and the inhibition was about the same in the peak. In contrast, the normal γ-globulin inhibited in the Gm(b) test system, whereas there was a definite decrease in inhibiting capacity in the peak. In this case

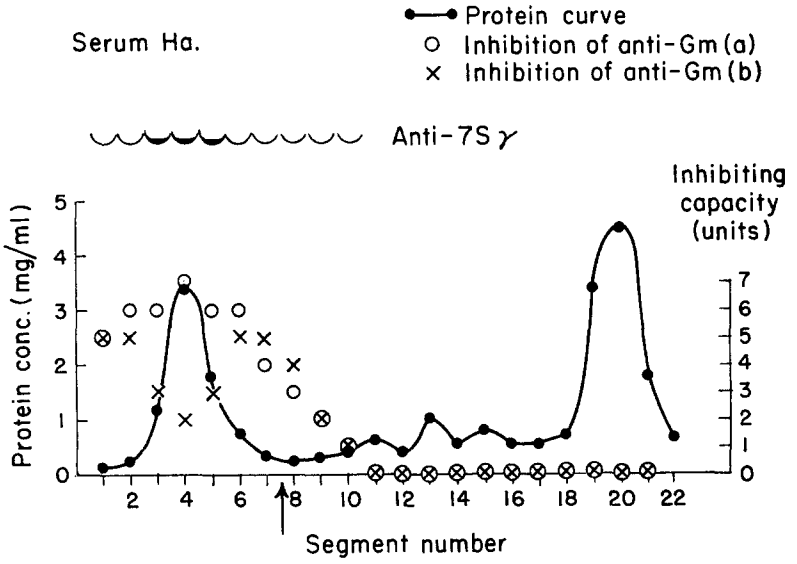

FIG. 4. Zone electrophoresis of serum Ha., showing that the myeloma protein and the normal γ-globulin on both sides of the peak are Gm(a+). The normal γ-globulin also is Gm(b+), whereas the myeloma protein is Gm(b−) as shown by the fall in inhibiting capacity in the segments directly under the peak.

the myeloma protein was regarded as Gm(a+b−). The slight inhibition in the Gm(b) test system by the segment which would be used as routine for typing (segment 4) could be accounted for by the contaminating normal Gm(b+) γ-globulin.

Thirty-six myeloma proteins of 7S γ-globulin type were tested for the Gm(x) character. Fig. 5 illustrates one which was Gm(a+x+); eleven were Gm(a+x−), and twenty-four Gm(a−x−). In nine cases of Gm(a+) myeloma proteins, both the peaks and the whole sera could be tested for the Gm(x) character. Only one serum was Gm(x+), the peak also being of this type. The Gm(x) character was not observed in the whole serum when it was absent in the isolated myeloma protein.

Seven sera were obtained from Negroes with 7S γ-myeloma proteins. Six of them were Gm(a+b+) in whole serum, and one Gm(a−b+). The latter type is not characteristic of Negroes and is probably due to admixture of white genes (20). This serum was therefore discarded from the material. The myeloma proteins isolated from the remaining six sera were of type Gm(a+b−). The routine myeloma protein preparation was clearly Gm(a+b−) in three cases. The zone electrophoresis of one of these sera is shown in Fig. 6, which demon-

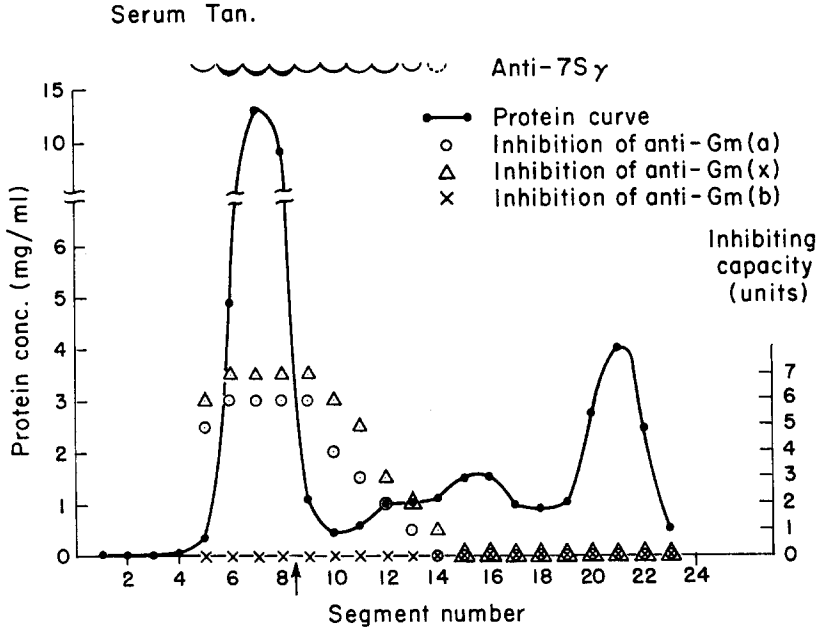

FIG. 5. Zone electrophoresis of serum Tan., showing that the myeloma protein and the normal γ-globulin ahead of the peak both are Gm(a+x+b−).

strates that there was inhibition of both anti-Gm(a) and anti-Gm(b) in the region ahead of the peak corresponding to the normal γ-globulin, whereas the protein in the peak inhibited anti-Gm(a) only. Three sera showed patterns similar to that of Fig. 4. There was some inhibition in the Gm(b) test system by the routine myeloma protein preparation. Inhibition experiments on a number of segments showed, however, that this inhibition could be accounted for by the contaminating normal Gm(b+) γ-globulin. All six Negro myeloma proteins were Gm-like (−). It is noted that the present test system for Gm-like (Carp/Warren) is unaffected by the Gm(b) type of the material to be tested. It could, therefore, be used to demonstrate the Gm-like factor in Gm(a+b−) Negro myeloma proteins. The original test system (Bomb./

Warren) would not be used since only Gm(b+) sera can be typed for Gm-like with these reagents.

Gm and Inv Factors in the Same Myeloma Protein and in Separate Fragments Produced by Papain Splitting.—Table III includes the Inv types of 28 isolated 7S γ-myeloma proteins. Three of them were Inv(a+b−) and 19 Inv(a−b+). Six possessed none of the factors and no preparation was positive for both factors. Inv factors were present both in group I and in group II 7S γ-myeloma proteins.

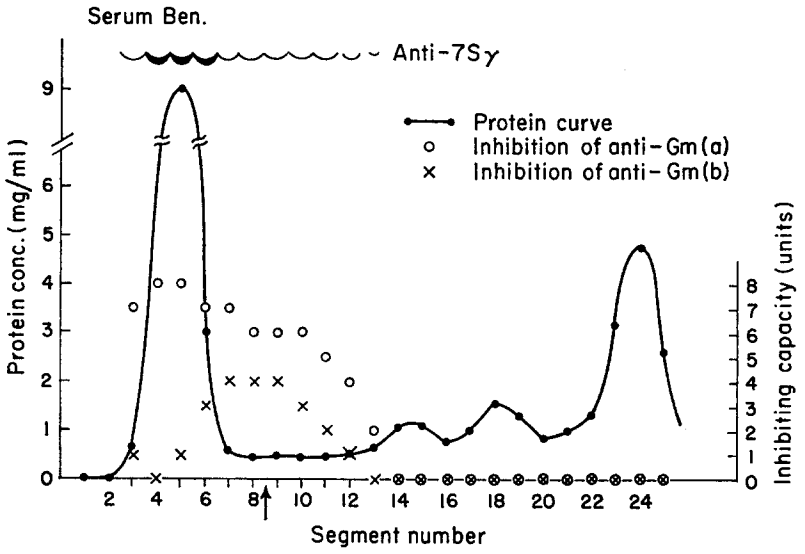

FIG. 6. Zone electrophoresis of serum from a Negro myeloma patient (Ben.). The normal γ-globulin ahead of the peak is Gm(a+b+), whereas the myeloma protein is Gm(a+b−).

In marked contrast to the presence of only one Gm or Inv factor in a single myeloma protein, when factors a and b are concerned, was the observation that many myeloma proteins were Gm(a+) and at the same time Inv(b+). In fact, the frequency distribution of the different Inv types was virtually identical in Gm(a+) and Gm(a−) myeloma proteins.

Three 7S γ-myeloma proteins of type Gm(a+) and Inv(b+) were split by papain at pH 7.4 and the fragments separated by zone electrophoresis on starch as previously described (31). In all cases, the Gm(a) determining site was found on the F fragment only, whereas the Inv(b) determining site was only found on the S fragment. These findings were identical with those made by similar splitting of Fr II γ-globulin made from pools of normal sera or electrophoretically isolated γ-globulin from individual normal sera. The Gm(a), Gm(x), and

Gm(b) factors were located on the F fragment and the Inv(a) and Inv(b) factors only on S.

Genetic Characters in β_{2A}-Myeloma Proteins.—Table V shows the Gm and Inv types of fifteen isolated β_{2A}-myeloma proteins. All of them were clearly Gm(a−b−), a finding confirming previous findings by Mårtensson (32), and Fahey and Lawler (33), indicating that Gm characters are not present in β_{2A}-globulins. However, in striking contrast, Inv(a) and Inv(b) characters were found in individual β_{2A}-myeloma proteins. The frequency distribution of the different Inv types was similar in β_{2A}- and 7S γ-myeloma proteins. Extensive experiments were made to rule out the possibility that contaminating 7S γ-globulin was responsible for the results. The fact that all preparations were Gm(a−b−) in itself made this interpretation improbable, and all other tests

TABLE V

Gm and Inv Types of Isolated β_{2A}-Myeloma Proteins

Gm(a+b−)	0	Inv(a+b−)	1
Gm(a+b+)	0	Inv(a+b+)	0
Gm(a−b+)	0	Inv(a−b+)	9
Gm(a−b−)	15	Inv(a−b−)	5
Number tested.....	15		15

showed that the contamination of 7S γ-globulin in the preparations was very small and could not account for the type results. Determination of inhibiting capacity of proteins eluted in the γ- and β-regions of starch blocks (*cf.* Fig. 2) showed that the serum γ-globulin might possess the Inv(b) factor when it was absent in the peak; in other instances it was present in both regions.

The β_{2A}-myeloma proteins often show signs of heterogeneity, both in man (1) and in transplantable plasma cell tumors in mice (5). Serum Lew. showed two sharp peaks with slightly different electrophoretic mobility in agar electrophoresis. Fig. 7 shows that a partial separation of the two peaks was obtained by zone electrophoresis on starch. All segments of the block lacked inhibiting capacity in the Gm(a), Gm(b), and Inv(b) test systems. The lack of inhibition in both Gm test systems apparently was due to the very low 7S γ-globulin concentration in this serum. This was borne out by very weak precipiting lines between the protein of segments 5 to 10 and rabbit anti-human 7S γ-globulin. The Inv(a) test system showed a clear difference between the two peaks: three segments from the first peak (segments 8 to 10) inhibited the agglutination completely down to a protein concentration of 0.12 mg/ml, segment 11 gave an intermediate pattern, and the segments corresponding to the second peak did not inhibit at a concentration of 1 mg/ml. It was concluded that the first peak was Inv(a+) and the second Inv(a−). This phenomenon of

genetic heterogeneity does not appear to be a general one. Two other β_{2A}-myeloma sera showing electrophoretic heterogeneity had constant activity throughout the peak regions in the Inv(a) and Inv(b) test systems.

Genetic Characters in 19S γ-Globulins and Urinary Bence Jones Proteins.— Six macroglobulins purified from sera of patients with Waldenström's macroglobulinemia were Gm(a−b−), again confirming Mårtensson's (32), and Fahey and Lawler's (33) findings that 19S γ-globulins lack Gm characters. However, two of them were clearly Inv(b+).

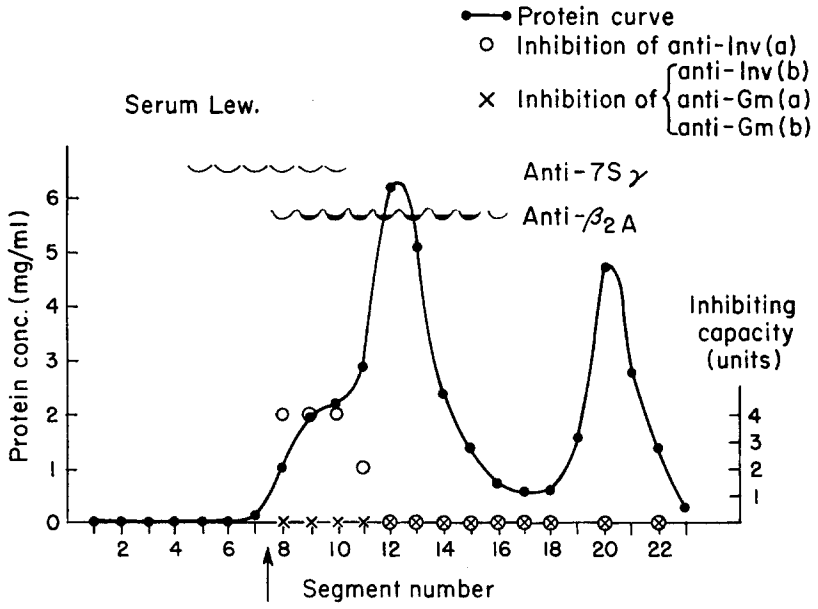

FIG. 7. Zone electrophoresis of a β_{2A}-myeloma serum (Lew.), showing two bands. Only the first of these (segments 8 to 10) is positive in the Inv(a) test system. The second peak (segments 12 to 14) is Inv(a−).

Eleven urinary Bence Jones proteins were tested. All were Gm(a−b−), whereas six of them possessed an Inv character. Table VI gives the data on Inv types of the isolated Bence Jones proteins as compared with those of the myeloma protein in the serum of the same patients. There was a striking correlation between the antigenic classification of Bence Jones proteins in groups I and II (29) on the one hand and the Inv types on the other. Four Bence Jones proteins were of group II, all being Inv(a−b−), whereas all but one of seven group I Bence Jones proteins had an Inv character.

Serum Spi. contained a myeloma protein of group II which was Inv(a−b+).

The Inv character was, nevertheless, not expressed in the urinary Bence Jones protein which was also of group II.

Serum Ro. contained a myeloma protein of group I which was Inv(a−b−). The Bence Jones protein was clearly Inv(a+b−), thus showing that a certain genetic character may be present in the Bence Jones protein even though it is lacking in the same patient's myeloma protein. The patient was genetically of type Inv(a+b+) since both these characters could be demonstrated in the normal γ-globulin which, by zone electrophoresis of serum, could be easily separated from the myeloma protein (Fig. 2 in reference 34).

TABLE VI

Inv Types and Serological Classification of 7S γ-Myeloma Protein and Urinary Bence Jones Protein from Eleven Patients

Patient	Myeloma protein		Bence Jones protein	
	Inv type	Group	Inv type	Group
Ger.	Inv(a−b+)	I	Inv(a−b+)	I
Car.	Not available		Inv(a−b−)	II
DeM.	Not available		Inv(a−b+)	I
Pat.	Inv(a−b+)	I	Inv(a−b−)	II
Cum.	No peak		Inv(a−b+)	I
Pro.	No peak		Inv(a−b−)	II
Haw.	Inv(a−b+)	I	Inv(a−b+)	I
Spi.	Inv(a−b+)	II	Inv(a−b−)	II
Ro.	Inv(a−b−)	I	Inv(a+b−)	I
Tan.	Inv(a−b+)	I	Inv(a−b+)	I
Sel.	Inv(a−b+)	I	Inv(a−b−)	I

DISCUSSION

The present studies on genetic characters in isolated myeloma proteins of various kinds have furnished considerable information concerning these characters in the corresponding proteins in normal sera, whereas no evidence indicated that the myeloma proteins are truly abnormal because of the malignant process. These investigations, presented in part previously (31, 34), confirmed the observations on myeloma proteins of Mårtensson (32), and Fahey and Lawler (33) that Gm-determining sites are present only on 7S γ-globulins, and showed that Inv-determining sites are present in addition on 19S γ-globulins, β_{2A}-globulins, and Bence Jones proteins. Certain similar findings were made by Franklin *et al.* (35).

After papain splitting of pooled human γ-globulin, γ-globulin from individual normal sera, and 7S γ-myeloma proteins (31), the Gm-determining sites were found only on the F fragment (36) and the Inv-determining sites only on the

S fragment. The results with three highly homogeneous myeloma proteins are particularly relevant since they strongly suggest that the same γ-globulin molecule contains the two sites in different fragments. Information on splitting of 7S γ-globulin molecules into so called heavy (H) and light (L) polypeptide chains by reduction and alkylation (37) along with the antigenic relationships between the H and L chains and the fragments produced by papain splitting (37 a) plus the occurrence of L chains in Bence Jones proteins (38), indicate that the Gm-determining site is present on the H chain and the Inv site on one of the L chains. They are therefore on different polypeptide chains within the 7S γ-globulin molecule. Previous data (31, 34) further indicate that there is a common genetic make-up of a part of 7S γ-globulin, 19S γ-globulin, and β_{2A}-globulin. This is particularly interesting since this part of the 7S γ-globulin molecule is known to contain the antibody combining site (39–41). The findings are in agreement with previous findings which indicate that the immunological cross-reaction between 7S γ-globulin and the other proteins is due to antigenic determinants present on the S fragment after splitting with papain, whereas antigenic determinants present on the F fragment of 7S γ-globulin appear not to be shared by the other proteins (41–44).

Current evidence indicates that 7S γ-globulins, 19S γ-globulins, and β_{2A}-globulins are produced by plasma cells or related cells in the normal individual, and genetic characters are demonstrable on all these proteins. In multiple myeloma, malignant cells of the plasma cell series produce the same kinds of proteins, and it is evident that the genetic factor Gm(a), Gm(b), Gm(x), Inv(a), and Inv(b) may be expressed in the tumor product. Genetic heterogeneity was demonstrable in the protein product of the tumor in two cases. In serum Lew., two sharp peaks were observed on agar electrophoresis. Both peaks consisted of markedly increased amounts of β_{2A}-globulin and gave reactions of identity with various rabbit antisera to human 7S γ-globulin and β_{2A}-globulin. Fig. 7 shows that the two peaks had different Inv types, one being Inv(a+) and the other Inv(a−). In case Ro., the myeloma protein in serum was a 7S γ-type protein of type Gm(a−b−), Inv(a−b−), whereas the urinary Bence Jones protein was Gm(a−b−), Inv(a+b−). These findings may indicate that in case Lew. two different clones of cells produce the two β_{2A}-globulins in serum, and in case Ro. that one clone of plasma cells produce the myeloma protein and another clone the urinary Bence Jones protein. Somewhat similar findings were made by Potter and Kuff (6) who observed that a transplantable plasma cell neoplasm in inbred mice produced two different kinds of proteins, one of γ-and the other of β-type immunologically. Two stable lines could be established by serial transplantation from a single host, one line producing γ-type and the other β-type protein, thus indicating the proliferation of two different clones of cells in the original host.

In heterozygous individuals it appears that no myeloma protein or Bence

Jones protein contains both factors determined by alternate alleles at a single locus. In patients whose normal γ-globulin was Gm(a+b+), the myeloma protein was found to be of type Gm(a+b−), Gm(a−b+), or Gm(a−b−), whereas both factors never appeared together in the myeloma protein. Similar findings were made by Mårtensson (32) who observed that three out of twenty-four 7S γ-myeloma proteins were Gm(b+) and all three lacked the Gm(a) character. Fahey and Lawler (33) indicated that some myeloma proteins were Gm(a+b+). However, when their figures are evaluated at equivalent protein concentrations, it appears that the findings may well be explained by contaminating normal Gm(b+) γ-globulin in the myeloma protein preparations. Since the frequency of the Gm(b) factor is so low in isolated myeloma proteins, the lack of Gm(a+b+) myeloma proteins might be fortuitous, although this appears improbable when adding up Mårtensson's (32) and the present data. In one case (*cf.* Table II), the isolated myeloma protein was Gm(a−b+) when the normal γ-globulin of the same serum was Gm(a+b+). The lack of the Gm(a) character in this myeloma protein is rather striking when one considers that in thirteen (65 per cent) of twenty cases the myeloma protein was Gm(a+) when the normal γ-globulin was Gm(a+).

A total of fifty-nine 7S γ-, 19S γ-, and β_{2A}-myeloma proteins and Bence Jones proteins were tested for the Inv factors; thirty-four of them (58 per cent) were Inv(b+) and five Inv(a+). If the presence of Inv(a) factor did not influence the chance of having Inv(b) in the same protein, one would expect that three of the Inv(a+) proteins (58 per cent) be Inv(a+b+) and two Inv (a+b−), whereas all were Inv(a+b−). The findings on Bence Jones protein Ro. appear to be particularly significant. Of seven group I Bence Jones proteins, all but one had an Inv character showing that there is a very strong tendency to express the genetic information of the individual to the Bence Jones protein if the latter is of type I. The type most frequently encountered was Inv(a−b+), which is to be expected since about 80 per cent of all Americans are of this type. The normal γ-globulin in serum Ro. was of type Inv (a+b+) showing that she was a heterozygote at this locus; the Bence Jones protein, however, contained only one of these characters being Inv(a+b−). These findings strongly suggest that in a Gm(a+b+) individual, the Gm(a) and Gm(b) characters are present on different γ-globulin molecules and produced by different cells. The same appears to be true for the Inv(a) and Inv(b) characters in an Inv(a+b+) individual. If they were on the same molecule, or were produced by the same cells, one would expect a large percentage of myeloma proteins to be of types Gm(a+b+) and Inv(a+b+). It appears therefore that a single clone of proliferating plasma cells has a restricted capacity to express its genetic information in its protein product: in the heterozygous individual only one of the genetic characters of a particular locus is expressed in the protein product.

Fig. 5 shows that a myeloma protein may be Gm(a+x+) and Mårtensson also found a myeloma protein of this type (32). This indicates that the Gm(a) and Gm(x) characters may be present on the same γ-globulin molecules in a Gm(a+x+) individual, or at least that they are both produced in the same cells. This might be expected since genetic evidence indicates that both Gm(a) and Gm(x) factors are the product of a single allele called Gm^{ax}, or in a slightly different interpretation by two genes, Gm^{a} and Gm^{x}, which are closely linked and present on the same chromosome.

The Gm(b) character was demonstrable in only two of forty-seven 7S γ-myeloma proteins. This low frequency is striking since the frequency of Gm(b+) individuals in the American white population is about 90 per cent (20). The explanation of the rare occurrence of the Gm(b) character is of course of considerable interest. Two main views may be considered: Firstly, the Gm(b) character may be present only on a small percentage of the γ-globulin molecules in a normal Gm(b+) individual. If the malignant process arises as a result of proliferation of one clone of cells, one might then expect that the Gm(b) character be expressed in the myeloma protein in only a small percentage of cases. Considerable indirect evidence appears to support this view, and this is discussed in detail in a separate publication (45). The evidence briefly is as follows: The inhibiting capacity of Gm(b+) myeloma proteins is significantly greater than that of similarly isolated Gm(b+) 7S γ-globulin from normal individuals (*cf.* Fig. 1 and reference 32). Isolated antibodies may not possess all of the genetic characters which are present in the total 7S γ-globulin of an individual; this is particularly striking for the Gm(b) character which often appears to be absent in anti-Rh 7S γ-globulin of Gm(b+) individuals (17, 46). The allotypes of rabbit γ-globulin (47) are very similar to the γ-globulin types in humans. A certain allotypic specificity appears not to be present on all γ-globulin molecules in a given rabbit. The frequency of γ-globulin molecules carrying a certain allotype specificity further varies for different specificities and is far from 100 per cent even in the homozygous state in some instances.

The second possibility to explain the deficiency of the Gm(b) character is of course that the proteins are truly abnormal and deficient because of the malignant process. This view appears considerably less likely; actually these studies have indicated an amazing similarity of the myeloma proteins and individual normal γ-globulins.

A study of isolated 7S γ-myeloma proteins from Negroes appeared promising as a means of stuying the character of the Gm(b) type in Negroes. In whites, a Gm(a+b+) individual is heterozygous Gm^{a}/Gm^{b} (17), the genes controlling the two factors being on different chromosomes. In Negroes, the genetic situation is markedly different in so far as all Negroes are Gm(a+b+) with the presumed genotype Gm^{ab}/Gm^{ab}. Here, both factors appear to be produced by a single allele on one chromosome (20). A highly surprising finding was the obser-

vation that all of six 7S γ-myeloma proteins from Negroes were Gm(a+b−). The low frequency of the Gm(b) factor in Negro myelomas indicate that the effect of gene *Gm*^{b} is very similar indeed in the two races. Previously, the data on Negroes might be explained by the hypothesis that the "Gm substance" in Negroes was peculiar, having the ability to cross-react in both Gm(a) and Gm(b) test systems. Such a hypothesis is excluded by the present findings since it would imply that all myeloma proteins from Negroes be Gm(a+b+).

SUMMARY

The genetic factors Gm(a), Gm(b), Gm(x), and Inv(a), Inv(b) described for normal human γ-globulin were all found in different myeloma proteins. A single myeloma protein never contained more than one product of alternate alleles even in heterozygous individuals. However, factors determined by the two different loci were often found in the same myeloma protein. The Gm(a) character of the myeloma protein parallelled that of the normal γ-globulin of the same serum in most cases. In contrast, the Gm(b) character was usually absent in the myeloma protein when it was directly demonstrable in the normal γ-globulin. The myeloma proteins from six Negroes were Gm(a+b−), whereas the normal γ-globulin was Gm(a+b+). This indicates that the effect of gene *Gm*^{b} is similar in Negroes and whites, even though its relation to gene *Gm*^{a} is different in the two races.

Gm factors were found only in the 7S γ-globulin type myelomas and not in other products of plasma cell tumors. Inv characters were, however, present in all four types of proteins studied, namely 7S and 19S γ-globulins, β_{2A}-globulins, and Bence Jones proteins. In two instances, genetic heterogeneity of the protein products was demonstrated suggesting the proliferation of more than one clone of plasma cells in some multiple myeloma patients.

The accumulated evidence obtained in this study strongly suggested that the presence and absence of genetic characters was compatible with the concept that myeloma proteins were closely analogous to individual moieties in the spectrum of normal γ-globulins rather than truly abnormal proteins. Their study offered evidence of a heterogeneity of genetic characters among the normal γ-globulins in a given individual. It also appears probable that in normal individuals single plasma cells have a restricted capacity to express genetic information in their protein product.

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