ON THE RELATIONSHIPS BETWEEN THE γ-GLOBULIN GENES OF THE GM SYSTEM*

A STUDY OF GM GENE PRODUCTS IN SERA, MYELOMA GLOBULINS, AND SPECIFIC ANTIBODIES WITH SPECIAL REFERENCE TO THE GENE Gm^f

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The genes Gm^a and Gm^b have generally been considered to be alleles in Caucasians (1–3). Studies of myeloma globulins and of isolated antibodies by several workers led to the conclusion that only a minor proportion of the γ_2-globulin molecules contain the expression of the gene Gm^b, even in the Gm^b homozygote, whereas the gene Gm^a partakes in the synthesis of a major fraction of the γ_2-globulin (4–9). A large proportion of the γ_2-globulin molecules in Caucasian individuals possessing the gene Gm^b seem to be Gm(a−b−).

Recently another hereditary group characteristic of human γ-globulin was identified and given the designation Gm(f) (10). Gm(f+) γ-globulin is detected by means of rare human sera containing anti-Gm(f). Gold made the initial observation that a serum that apparently gave the same results as an anti-Gm(b) when used to type whole sera agglutinated some anti-Rh–coated red cells that were not agglutinable by anti-Gm(b). The agglutinator of this serum was subsequently named anti-Gm(f) (10). Other genetic factors of human γ-globulin that have recently been reported are Gm (b^w), (b^α), (b^β), (b^γ), (e), and (p), but Gm(f) does not seem to be identical with any of these (10).

The characteristic Gm(f) was generally found to be inherited together with Gm(b) in the Caucasians studied. It was considered possible that Gm(f) might correspond to a gene involved in the synthesis of the Gm(a−b−) γ_2-globulins found in Gm(b+) white individuals. This study is concerned with the definition of Gm^f and with the relationships between genes Gm^f, Gm^a, Gm^b, and their products.

Material and Methods

Gm Determinations

Agglutination Inhibition Tests.—

Principle: Some human sera agglutinate Rh-positive red cells coated with incomplete anti-Rh antibodies. The agglutinator can sometimes be shown to react with a genetic γ-globulin

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determinant. Sera from individuals whose γ-globulin contains the specific determinant inhibit the agglutination. For example, a Gm(a) typing system consists of an anti-Gm(a) agglutinator and red cells coated with Gm(a+) anti-Rh antibodies; Gm(a+) sera inhibit the agglutination, while Gm(a−) sera do not.

Reagents used: For all the Gm factors studied, *i.e.* Gm(a), (b), (f), and (x), typing systems were used in which the agglutination was inhibited by Gm(+) sera diluted 1/100 or more but not by even undiluted Gm(−) sera.

1. Anti-Gm(f): Two sera, Mau. and A. J., were used (10). Both were from donors without signs of rheumatoid arthritis. On sucrose density gradient ultracentrifugation the agglutinators in the 2 sera sedimented like 19S γ-globulins. They gave identical results when used for typing of 10 Negro sera, 15 Eskimo sera, and 30 Caucasian sera, including the Gm(b+f−) and Gm(b−f+) sera encountered; both of them agglutinated in parallel red cells coated with various anti-Rh antibodies that did not coat for anti-Gm(a) or for anti-Gm(b); and both reacted with determinants recovered in the S fraction after papain digestion or γ-globulin (11).

2. Anti-Gm(b): Most determinations were made with serum I. G. which contains an anti-Gm(b) sedimenting like 7S γ-globulin (see reference 12). Five other anti-Gm(b) sera were used to type some of the Gm(a−b+f−) myeloma globulins and gave concordant results.

3. Anti-Gm(a)*:* One agglutinator from a healthy donor, B. A., was used for most determinations. It gave identical results with 3 other anti-Gm(a) agglutinators (1 of which was of 7S type) in typings of 10 myeloma globulins of various Gm types, 15 Eskimo, 10 Negro, and 20 Caucasian sera.

4. Anti-Gm(x): One serum from a non-rheumatoid subject, R. A., was used.

5. Anti-Inv(l) and anti-Inv(a): Two anti-Inv(l) sera were provided by Dr. Erna v. Loghem, Amsterdam, Netherlands, and 1 by Dr. A. G. Steinberg, Cleveland. The anti-Inv(a) used was the original serum, Virm., of Dr. Ropartz, Rouen, France.

Technique: 0.06 ml of the sample to be tested, 0.03 ml of the anti-Gm serum in a suitable dilution, and 0.03 ml of anti-Rh–coated red cells in a 2 per cent suspension were mixed in round-bottom test tubes. After standing for 1 hour at room temperature the tubes were centrifuged for 45 seconds (serofuge, Clay-Adams Inc., New York) and then read immediately for agglutination with the naked eye. Isotonic phosphate-buffered NaCl (pH 7.2) was used as a diluent. Conventional controls were included in all determinations.

Most of the 620 Swedish sera were typed in 1 dilution, 1/15, in the Gm(a), (b), and (f) systems. The Negro sera, as well as 100 of the Swedish sera, were typed in the 2 dilutions 1/5 and 1/30, in both of which the results were always identical.

The anti-Rh sera were typed in the dilutions 1/10, 1/20, and 1/40. Two special controls were included: (*a*) serum 1/10 + anti-Gm + uncoated cells and (b) serum 1/10 + saline + coated cells. These controls were always negative.

The myeloma sera and myeloma globulin preparations were tested in series of twofold dilutions to determine the inhibitory titres in the different Gm typing systems. Pipettes were changed between the dilution steps.

Agglutination Tests.—Some experiments were done to determine the Gm types of the anti-Rh antibody molecules of certain sera. Tables VI *a* and VI *b* refers to an experiment in which 10 volumes of each anti-Rh serum diluted ½ was mixed with 1 volume of packed Rh-positive red cells. After incubation at 37°C for 1 hour and repeated washing of the cells, the latter were tested for agglutinability by anti-Gm sera with a technique analogous to that described above.

The experiment referred to in Table VII was done in a similar way, except that 1 volume of a 20 per cent suspension of cells was coated with 4 volumes of different dilutions, given in the table, of the anti-Rh sera.

Myeloma Sera and Myeloma Globulin Preparations

24 myeloma sera had been obtained from Dr. R. Bachmann, Dr. C.-B. Laurell, and Dr. U. Nilsson, the Department of Clinical Chemistry, Malmö General Hospital, Malmö, Sweden. Routine paper electrophoresis, starch gel electrophoresis, and immunoelectrophoresis, employing specific anti-γ_2, anti-γ_1A, and anti-γ_1M-antisera, had been performed there. All sera contained myeloma globulins of immunological γ_2-type. The concentrations of the myeloma globulins had been determined by paper electrophoresis and are given later on in Table V together with the results on each serum. Only sera with myeloma peaks above 35 mg/ml were included. The levels of normal γ-globulin (non-myeloma γ-globulin) were depressed in all sera. The estimated amount of normal γ-globulin in these sera never exceeded 5 mg/ml.

Immunoelectrophoresis was run also in our laboratory on those myeloma sera that contained Gm(a−b−f−) myeloma globulin. Specific anti-γ_2-, anti-γ_1A-, and anti-γ_1M-rabbit antisera were used (Behringwerke A.G., Marburg-an-der-Lahn, Germany).

Of the 18 purified myeloma globulin preparations, 14 were obtained from the Department of Clinical Chemistry, Malmö General Hospital, and 4 from Dr. H. G. Kunkel, The Rockefeller Institute. The procedures used for purification of the 14 preparations from Malmö have been described in references 8 and 13, that for the 4 preparations from The Rockefeller Institute in reference 6. All these preparations had been judged as containing only traces of residual normal γ-globulin. Gm(a) and (b) types of 4 of these were published in reference 4, types of 10 in reference 8, and types of 4 in reference 6.

Sera from Caucasians and Negroes

The Swedish sera (Table I) had been sent to our institute for routine serological tests. Around half were from normal blood donors and half from patients of a Swedish general hospital.

The 10 American Negro sera (Table I) were received from Dr. A. G. Steinberg, Western Reserve University, Cleveland.

Anti-Rh Sera

The incomplete anti-Rh sera used were gifts from several laboratories. Two of the sera identified in Table VI *b* have been used by other groups studying the Gm factors. M. B. was received from Dr. C. K. Osterland, The Rockefeller Institute, and 199 from the State Serum Institute, Copenhagen (equals their serum 423199).

RESULTS

Sera.—

Caucasians: Table I, *A*, gives the Gm(a), (b), and (f) types of 560 Swedish sera.

In addition 60 Swedish sera, in which types Gm(a+b−) and Gm(a+b+) were intentionally overrepresented were typed for Gm(f). All of 26 Gm(a+b−) sera proved to be Gm(f−). Of 26 Gm(a+b+) sera 1 was Gm(f−) and the rest Gm(f+). All 8 Gm(a−b+) sera were found to be Gm(f+).

Thus, of 620 Swedish sera tested all except 3 were Gm(b+f+) or Gm(b−f−); 2 were Gm(b+f−) and 1 was Gm(b−f+). These 3 sera have been tested repeatedly and with both of the anti-Gm(f) agglutinators. 2 of 3 sibs of a Gm(a+b+f−) individual were also Gm(a+b+f−).

Negroes: Of 10 American Negroes (Table I, *B*) 7 were Gm(a+b+f−) and 3 were Gm(a+b+f+).

Myeloma Globulins.—The γ_2-myeloma globulin in 24 sera and in 18 purified myeloma globulin preparations from Caucasian individuals were grouped with respect to Gm(a), (b), (f), and (x) (Tables II to IV).

TABLE I

Gm Types of 560 Swedish Sera and 10 American Negro Sera

Sera	No.	Per cent
A. Swedish		
Gm(a+b+f+)	248	44
Gm(a−b+f+)	235	42
Gm(a+b−f−)	75	13
Gm(a+b+f−)	1	<1
Gm(a+b−f+)	1	<1
Total	560	
B. American Negro		
Gm(a+b+f+)	3	
Gm(a+b+f−)	7	
Total	10	

When typed with respect to a particular Gm factor, *i.e.* Gm(a), (b), (f), or (x), each myeloma serum (or myeloma globulin preparation) fell into one or another of the following categories:

A. Sera without the specific activity: *Gm(−) normal and myeloma globulin.*

B. Sera with activity approximately equal to that of normal Gm(+) sera (or lower and then proportional to the decreased concentration of the normal γ-globulin): *Gm(+) normal and Gm(−) myeloma γ-globulin.*

C. Sera with activity considerably higher than that of normal Gm(+) sera: *Gm(+) myeloma globulin.*

As an example, the results of Gm(a) determinations on the myeloma sera are given in Fig. 1. The 6 myeloma sera which were Gm(a−) are not included. Of the 18 Gm(a+) myeloma sera 7 constitute 1 group (hatched columns). They had inhibitory titres that were on the average slightly lower than those of normal Gm(a+) sera (Fig. 1, upper diagram). These titres corresponded to the decreased concentrations of normal γ-globulin. The other 11 (filled columns) had considerably higher inhibitory titres than Gm(a+) normal sera (Fig. 1, upper diagram). Therefore the normal γ-globulin cannot account for more than a minor fraction of the Gm(a) activity; it must instead be

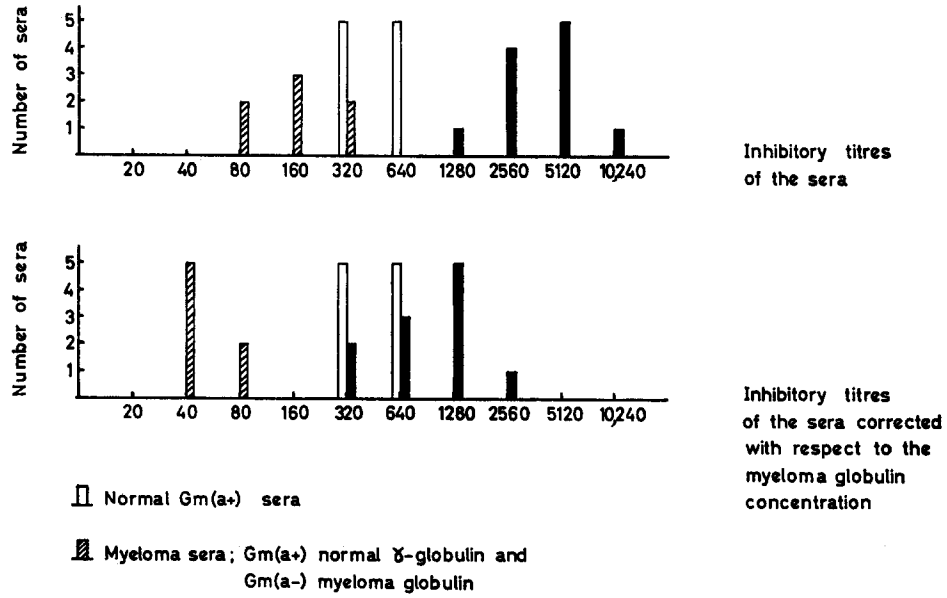

FIG. 1. Gm(a) determinations on myeloma sera. Distribution of inhibitory titres in the Gm(a) test system for 18 Gm(a+) myeloma sera and for 10 Gm(a+) normal reference sera.

In the *upper diagram* the myeloma sera have been diluted in the ordinary way; *i.e.*, the dilutions indicated are the true dilutions of the sera.

In the *lower diagram* the inhibitory titres have been *corrected* with regard to the myeloma globulin concentrations; *e.g.*, the inhibitory titre of a myeloma serum that contains myeloma γ-globulin in a concentration 4 times as high as the concentration of γ-globulin in normal sera, has been reduced by a factor of 4. Thus, the dilution designation 1/1 would refer to a myeloma globulin concentration of 10 mg/ml as that is the approximate concentration of γ-globulin in normal sera.

Myeloma sera which were entirely Gm(a−) have not been included.

The inhibitory titres given in the diagrams represent the average of 3 determinations. The difference in titre between 2 determinations on 1 serum was at most 1 titre step.

ascribed mainly to *Gm(a+) myeloma globulins*. The Gm(a) titres can be explained, partly by the high concentrations of the myeloma globulins and partly by a higher activity per unit of protein for the myeloma globulins than for the whole γ-globulins of normal Gm(a+) sera (Fig. 1, lower diagram).

All sera and preparations examined contained at least 6 to 8 times as much myeloma globulin as normal γ-globulin (see Material and Methods and Table V). As exemplified, this made it possible to decide by quantitative considerations whether a certain Gm activity demonstrated in the serum or the preparation should be ascribed to the myeloma protein or to the residual normal γ-globulin; in other words, sera of categories B and C were readily distin-

guished from one another. The distinction was facilitated still more by the high specific activity of myeloma globulins Gm(+) in respect of a particular Gm factor.

First, 18 myeloma globulins (purified myeloma globulin fractions) of known Gm(a) and (b) types were grouped in the Gm(f) system (Table II). They had been selected to include as many of the rare Gm(b+) myeloma globulins

TABLE II

Gm(f) Types of 18 Myeloma Globulins Previously Typed for Gm(a) and Gm(b), and Selected to Include as Many Gm(b+) as Possible

Type	(f+)	(f−)
Gm(a−b+)	0	9
Gm(a+b−)	0	4
Gm(a−b−)	4	1

TABLE III

Gm(a), (b), and (f) Type Frequencies among 24 γ_2 (7Sγ)-Myeloma Globulins and among Swedish Sera (i.e., Whole γ-Globulins from Normal Individuals)

Type	Myeloma globulins, No.	Sera
		per cent
Gm(a+b+f+)	—	44
Gm(a+b+f−)	—	<1
Gm(a+b−f+)	—	<1
Gm(a−b+f+)	—	42
Gm(a+b−f−)	11	13
Gm(a−b+f−)	—*	—
Gm(a−b−f+)	10	—
Gm(a−b−f−)	3	—

* Although type Gm(a−b+f−) is missing in these 24 myeloma globulins (not selected on the basis of Gm type), it does occur (see also Table II).

as possible in the hope that the latter would provide critical information on the relationship between Gm(f) and Gm(b). All 9 Gm(b+) myeloma globulins proved Gm(f−).

Second, 24 myeloma sera were studied. The only criteria of selection for these were that they should contain myeloma globulin of immunological γ_2-type in concentrations above 35 mg/ml and decreased amounts of normal γ-globulin. The distribution of the Gm(a), (b), and (f) types among the myeloma globulins is given in Table III.

The following points deserve special attention: (*a*) No myeloma globulins were positive for more than 1 of the factors Gm(a), (b), and (f), while about as many as 90 per cent of whole sera were positive for 2 or all 3 factors. In other words, none of the myeloma globulins appeared to contain the product or more than 1 of the 3 genes Gm^a, Gm^b, and Gm^f, whereas the total γ-globulin of most individuals contains the product of 2 or of all 3 of these genes. (*b*) The types Gm(*a*+b−f−) and Gm(a−b−*f*+) represented the two main groups of the γ_2-myeloma globulins; each comprised roughly 40 per cent. The types Gm(a−*b*+f−) and Gm(a−b−f−) were minor groups.

Immunologically the 3 Gm(a−b−f−)myeloma globulins were, like the other 21, of γ_2 (7Sγ)-type. This was established by immunoelectrophoresis (see Material and Methods).

TABLE IV

Gm(x) Types of 24 γ_2-Myeloma Globulins (the Same as in Table III)

Type	(x+)	(x−)
Gm(a+b−f−)	5	6
Gm(a−b+f−)*	—	—
Gm(a−b−f+)	—	10
Gm(a−b−f−)	—	3

* The 9 myeloma globulins of type Gm(a−b+x−), which are not included in this table, were all Gm(x−).

The (relatively) homogeneous myeloma globulins were generally more strongly inhibitory than the heterogeneous whole γ-globulin from normal individuals positive with respect to the same factor: (*a*) The inhibitory activity of Gm(b+) myeloma globulins was 4 to 32 times as high as that of whole γ-globulins from normal Gm(b+) individuals. (*b*) The Gm(f+) myeloma globulins, on the other hand, were on the average only about twice as inhibitory per unit protein as whole γ-globulins from normal Gm(f+) persons. For Gm(a) the corresponding ratio was also roughly 2:1 (Fig. 1).

The myeloma globulins of all 42 sera and preparations were grouped with respect to Gm(x). Six of them were Gm(x+), the remaining 36 Gm(x−). All 6 Gm(x+) myeloma globulins were also Gm(a+). Of the 24 myeloma globulins that had not been selected according to Gm type, 11 were Gm(a+). Five of these 11 were Gm(x+) (Table IV). The Gm(x+) myeloma globulins were equally or at most 2 to 4 times as inhibitory per unit of protein as whole γ-globulin from Gm(x+) normal individuals.

Table V gives detailed data for each of the 24 myeloma sera. Due to scarcity of material Gm(f) and Gm(x) determinations were not performed on all sera in the dilution range permitting grouping of the normal γ-globulin.

The myeloma sera and myeloma globulin preparations were initially investigated with anti-Gm(f) Mau. Each myeloma serum or preparation could be assigned to one of the 3 above-mentioned categories, A, B, or C.

Always when a serum had been typed as Gm(f−) with Mau. the same result was obtained with A. J. (category A), and when the Gm(f) activity could be accounted for by the normal γ-globulin, the 2 anti-Gm(f) sera gave parallel results (category B). Those myeloma

TABLE V

Gm Groups of the Myeloma Globulins and of the Normal γ-Globulins in the Same Sera

Designations of the myeloma sera	Myeloma globulin				Normal γ-globulin		Conc. of the myeloma globulin in the serum
	Gm(a)	Gm(x)	Gm(b)	Gm(f)	Gm(a)	Gm(b)	
							mg/ml
621	+	+	−	−	+	+	41
707	+	+	−	−	+	+	35
712	−	−	−	+	+	+	63
722	+	−	−	−	+	+	86
723	+	+	−	−	+	+	71
743	−	−	−	−	+	(+)	37
745	−	−	−	+	−	+	48
786	+	−	−	−	+	+	65
789	−	−	−	+	+	+	50
814	+	−	−	−	+	+	96
818	−	−	−	+	−	+	65
823	+	+	−	−	+	−	43
824	+	−	−	−	+	+	52
827	−	−	−	−	−	+	37
831	+	−	−	−	+	+	47
844	−	−	−	+	−	+	47
846	−	−	−	+	+	+	62
879	−	−	−	−	−	+	46
887	−	−	−	+	+	+	88
888	−	−	−	+	+	+	48
902	+	−	−	−	+	−	39
903	−	−	−	+	−	+	37
904	−	−	−	+	+	+	68
908	+	+	−	−	+	−	44

globulins that had been typed as Gm(f+) with Mau., because they inhibited 1 to 4 times as strongly as whole γ-globulin from normal Gm(f+) individuals (category C), gave unexpected results with A. J. Four of 10 myeloma globulins defined as Gm(f+) by Mau., also inhibited A. J. more strongly than normal whole Gm(f+) γ-globulin, but the other 6 were less inhibitory. However, the inhibitory titres of these 6 sera were higher than what could be accounted for by the normal γ-globulin. These myeloma globulins appeared to be Gm(f+) also as defined by A. J. though their activity was lower than that of some normal Gm(f+) γ-globulin molecules. The molecular basis and the significance of this observation is the subject of continued studies.

TABLES VI *a* AND VI *b*

Gm(a), (b), and (f) Types of Anti-Rh Antibodies

TABLE VI *a*

Anti-Rh Sera Selected Only for High Titre in Coombs' Test, but Not on the Basis of Gm Type

Gm type of the sera..	(a+b−f−)		(a+b+f+)				(a−b+f+)			
Serum designation...	1	2	3	4	5	6	7	8	9	10
Anti-Gm(a)										
B. A.	++	++	++	++	++	−	−	−	−	−
Stang.	++		++	++	++	−	−	−	−	−
Anti-Gm(b)										
I. G.	−	−	−	(−)	(−)	−	++	++	−	−
Berg.	−		−	−	−	−	+	+	−	−
Garv.	−		−	−	−	−	(+)	(+)		−
Anti-Gm(f)										
Mau.	−	−	++	++	++	++	(+)	++	++	++

TABLE VI *b*

Sera Selected because They Contain Anti-Rh Antibodies with Different Characteristics with Respect to Gm(a) and (b)

	Columns							
	I				II		III	
Gm type of the sera	a+b+ f+	a−b+ f+	a+b+ f+	a−b+ f+	a+b+ f+	a+b+ f+	a−b+ f+	a−b+ f+
Serum designation.....	199	M. B.	108	Car.	SB	602	696	418
Anti-Gm(a)								
B. A.	−	−	−	−	++	++	−	−
Stang.	−	−	−	−	++	++	−	−
Anti-Gm(b)								
I. G.	++	++	++	++	+	−	−	−
Berg.	++	++	++	++		−	−	−
Garv.	++	++	−	++		−	−	−
Anti-Gm(f)								
Mau.	−	−	++	++	++	++	++	++

Column I, anti-Rh sera found useful for Gm(b) typing systems; Column II, useful Gm(a) coats; Column III, sera with high titres in Coombs' test that do no render red cells agglutinable by anti-Gm(b), nor by anti-Gm(a); see Table VII.

As to the performance of the experiment given in Tables VI *a* and VI *b* see Material and Methods. The sign, ++, denotes agglutination of maximal strength, the sign, −, denotes no agglutination.

Anti-Rh Antibodies.—Eighteen strong incomplete anti-Rh sera (titres above 128 in Coombs' test) were used to coat Rh-positive red cells. The coated cells were tested for agglutinability with anti-Gm(a), (b), and (f) sera (Tables VI *a* and VI *b*).

When an anti-Rh serum has been used to coat Rh-positive red cells and the cells have been washed, the anti-Rh antibodies can be regarded as "isolated." Agglutination of these cells by, say, an anti-Gm(a) serum indicates that some of the antibodies are Gm(a+), while non-agglutination indicates that none, or only relatively few, of the antibodies are Gm(a+).

Judging from the agglutination reactions, the anti-Rh antibodies of Gm(b+f+) sera were most often Gm(f+), whereas they were very often Gm(b−). The anti-Rh antibodies of Gm(a+) sera were usually Gm(a+). It may also be noted that the anti-Rh antibodies of Gm(a+f+) sera most often contained both Gm(a) and Gm(f).

Though, *e.g.* sera 199 and 696 (see Tables VI *b*, and VII) provide clear instances of predominantly Gm(b+f−) respectively Gm(b−f+) anti-Rh antibodies occurring in Gm(b+f+) sera, it should be remarked that the former type is rare. Serum 199 has been selected from a large number (50 to 100) of strong anti-Rh sera and M. B. has been sent to us as being particularly useful in Gm(b) typing systems. Most Gm(b+f+) anti-Rh sera-coated red cells effectively for anti-Gm(f) but poorly or not at all for anti-Gm(b) (Table VI *a*).

Red cells coated with the 2 sera, M. B. and 199, which contain mainly Gm(b+f−) anti-Rh antibodies, were definitely more strongly agglutinated by anti-Gm(b) than cells coated with any other anti-Rh antibodies; including *e.g.*, the Gm(b+f+) anti-Rh antibodies of sera I. K. and Car. This difference was striking when the agglutination tests were performed with a less sensitive technique; *e.g.*, on slides or with aged cells. An increase in the amount of serum used to coat a certain quantity of red cells did not change the result in these experiments, which indicates that the anti-Rh antibodies were present in excess during the coating (see Discussion).

In previous studies (14) the anti-Rh antibodies of many anti-Rh sera appeared to lack the products of known Gm genes. Five (Nos. 6, 9, 10, 418, and 696) of the sera studied in the present investigation contained mainly Gm(a−b−) anti-Rh antibodies. In all 5 instances the antibodies were clearly Gm(f+).

Table VII gives the result of an experiment with 3 anti-Rh sera, 696, 199, and S. W., performed before anti-Gm(f) reagents were available. The result clearly shows that in these instances inability of an anti-Rh serum positive for a certain Gm factor to sensitize the cells to the corresponding anti-Gm was not caused by too low a concentration of antibodies during the coating. As only a limited number of anti-Rh antibodies can attach themselves to a cell these data do not make possible any exact estimate of the proportion of

TABLE VII

Experiment Showing That the Products of a Gm Gene Possessed by an Individual May Be (at Least Largely) Lacking among His Anti-Rh Antibodies

Anti-γ-globulin reagent used to test the coated cells	Dilution of anti-Rh serum								
	5	10	20	40	80	160	320	640	1280
Anti-Rh 199. Type of the serum: Gm(a+b+)									
Anti-Gm(a)	−	−	−	−	−	−	−	−	−
Anti-Gm(b)	+	+	+	+	−	−	−	−	−
Rabbit anti-human globulin	+	+	+	+	+	+	+	−	−
Anti-Rh S. W. Type of the serum: Gm(a+b+)									
Anti-Gm(a)	+	+	+	+	−	−	−	−	−
Anti-Gm(b)	−	−	−	−	−	−	−	−	−
Rabbit anti-human globulin	+	+	+	+	+	+	+	−	−
Anti-Rh 696. Type of the serum: Gm(a−b+)									
Anti-Gm(a)	−	−	−	−	−	−	−	−	−
Anti-Gm(b)	−	−	−	−	−	−	−	−	−
Rabbit anti-human globulin	+	+	+	+	+	+	+	−	−

Three anti-Rh sera were used to coat Rh-positive cells. For the coating each anti-Rh serum was used in 9 dilutions (1/5 to 1/1280). The coated cells were tested with anti-Gm(a), anti-Gm(b), and a rabbit anti-human globulin serum. The anti-Gm sera were used in 2 different dilutions with a fourfold concentration difference. Both dilutions gave identical results indicating the presence of anti-Gm in excess. A 2nd anti-Gm(a) and a 2nd anti-Gm(b) gave closely similar results.

Serum 199 coated only for anti-Gm(b), although the whole serum was Gm(a+b+); serum SW, only for anti-Gm(a); and serum 696 neither for anti-Gm(a) nor for anti-Gm(b). The latter coats effectively for anti-Gm(f). (See Table VI *b*.)

them that carries a particular Gm-specificity. But, still, it seems obvious that at most a minor percentage of the anti-Rh antibody molecules can be Gm(a+) in serum 199 or Gm(b+) in serum 696.

The Inv(a) and (l) type of the anti-Rh antibodies in 3 Inv(a+l+) sera were studied in some detail. Dr. Erna van Loghem, Amsterdam, who provided 2 of these sera (Nos. 2126 and 2264), had found 1 useful for Inv (l) typing systems and the other for Inv(a) typing systems. Dr. A. G. Steinberg who furnished the 3rd anti-Rh (Roehm) used it for Inv(a) and for Inv(l) systems.

The following experiment was done. The technique and the principle was the same as in the experiment referred to in Table VII: Red cells of group O Rh-positive were coated with each of the 3 sera in different dilutions. The coated cells were subsequently tested with anti-Inv(a) and anti-Inv(l) sera. The highest dilution of each anti-Rh serum that rendered the cells agglutinable by anti-Inv (1) respectively anti-Inv(a) was determined. A fourfold increase in the concentration of the anti-Inv(a) or anti-Inv(l) used to test the coated cells did not affect the result, which indicates that an excess of agglutinator was present. Three anti-Inv(l) sera gave identical results.

It proved that the ratio between the amount of anti-Rh serum needed to render the cells agglutinable by anti-Inv(a) and the amount needed to render them agglutinable by anti-Inv(1) was widely different for these 3 Inv(a+l+) sera. For the 1st serum the ratio was greater than 16:1, it coated for anti-Inv(l) in the dilutions up to 1/16 but not for anti-Inv(a) even undiluted; for the 2nd anti-Rh serum the ratio was 1:8 and for the 3rd, 3:1. In other words, the 2nd serum differed from the 1st by a factor of at least 128 and from the 3rd by a factor of about 24. These differences may be explained by the assumption that Inv(a) and Inv(l) do not cooccur in the same molecules in Inv(a+l+) sera.

DISCUSSION

In recent years studies of γ-globulins produced by 1 or by a few clones of plasma cells, myeloma globulins, and certain antibodies (4–9, 14–16), have yielded valuable information on the Gm genes and on γ-globulin synthesis in individual cells. Such investigations also proved essential for clarifying the relationship between Gm^f and the other γ-globulin genes.

Previous Studies of Gm Specificities in Isolated Myeloma Globulins and Specific Antibodies and the Gap in Our Knowledge Filled by the Information on Gm(f).— In extensive family and population studies on Caucasians Gm^a and Gm^b behaved like alternate alleles (1–3). The frequency of Gm^a is about 0.35 and of Gm^b about 0.65 in Northern European populations (1).

Though about 45 per cent of normal individuals possess both the gene Gm^a and the gene Gm^b, myeloma globulins have never been found to be Gm(a+b+). Most γ_2 (7Sγ)-myeloma globulins are Gm(a+b−) or Gm(a−b−), and only some 10 per cent Gm(a−b+) (4, 6–8). There is evidence that also among normal γ-globulin molecules types Gm(a+b−) and Gm(a−b−) are common and type Gm(a−b+) rare. For example, antibodies with a single antibody specificity in Gm(a+b+) sera are often largely Gm(a+b−) or, though more rarely, Gm(a−b+) (5, 9); and it is common that anti-Rh and other γ_2-antibodies from Gm^b homozygotes are largely Gm(a−b−) (9, 14). It has hitherto been obscure what gene(s), if any, take the place of Gm^b (or Gm^a) in the synthesis of the Gm(a−b−) γ_2-globulins.

In this study the majority of Gm(a−b−) myeloma globulins proved to be Gm(f+). Also, anti-Rh antibodies that had been classified as Gm(a−b−) were

generally found to be clearly Gm(f+). These findings indicate that Gm^f takes part in the synthesis of most Gm(a−b−) γ_2-globulins in Caucasians.

The Gm(a), (b), and (f) Types of γ-Globulin Molecules and of the γ-Globulin Produced by One or a Few Cell Clones.—A myeloma globulin was never found to contain the product of more than one of the genes Gm^a, Gm^b, and Gm^f (Tables II and III). This finding indicates,—for there are convincing reasons to believe that, at least with respect to Gm factors, most myeloma globulins represent *essentially* normal γ-globulins in abnormal concentration (see below),—that Gm(a), (b), and (f) do not (at least not as a rule) co-occur in the same molecule. They further show that these three types of γ_2-globulins *can* be made, independently, in different cells (even in individuals possessing two or all three of the genes).

The frequencies of the Gm types among myeloma globulins (Table III) may reflect the frequencies of these types among γ_2-globulin molecules in normal individuals. If so, large proportions of the γ_2-globulins of normals would be Gm(a+) or Gm(f+), whereas only a relatively small proportion would be Gm(b+) (see Table III). Genes not yet identified might be involved, in place of the Gm genes studied, in the synthesis of some 10 per cent (3 of 24 with due allowance for statistical variation) of the γ_2-globulin molecules.

Myeloma globulins positive for a given Gm factor generally had a higher activity per unit of protein than whole γ-globulin from Gm(+) persons. This difference is, in all probability, due to the greater homogeneity of the myeloma globulins. Only a proportion of the γ_2-globulin of a normal individual carries the factor and this proportion is presumably equal to the quotient between the activity of his γ-globulin and the activity of the Gm(+) homogeneous myeloma globulins. Also according to this line of thought only a minor fraction of the γ-globulin of normal individuals contains the products of the Gm^b gene (see also 4, 6–8), whereas major fractions carry Gm(a) or Gm(f) (see Results p. 1175).

In the majority of Gm(a+) sera the anti-Rh antibodies were clearly Gm(a+). Similarly, the anti-Rh antibodies of Gm(f+) sera were most often also Gm(f+). In contrast, only a minor proportion of Gm(b+) anti-Rh sera sensitized the cells to anti-Gm(b). Also this indicates that among the γ-globulin molecules the Gm(a+) and the Gm(f+) predominate over the Gm(b+).

Myeloma Globulins as Representatives of Normal γ-Globulins.—Different independent lines of reasoning support the major conclusions drawn concerning Gm genes and γ-globulin synthesis in normals: (*a*) The frequencies of the Gm types among normal γ-globulin molecules were estimated, first on the basis of the type frequencies among myeloma globulins and, second by comparing the specific activity of Gm(+) myeloma globulins and of whole γ-globulins from Gm(+) normal individuals. The estimates arrived at were concordant. (*b*) The frequency distribution of the Gm types among anti-Rh antibodies agrees strikingly well with their frequencies among myeloma globulins.

On the Mechanism of the Inactivity of Gm Genes in Individual Plasma Cells.—Chromosome abnormalities in plasma cell neoplasias of humans and of mice have been found by several workers, but instances of plasma cell myeloma with apparently normal karyotypes have also been reported (see references 17, 18). It might be appropriate to consider whether elimination of some of a given individual's Gm genes from single plasma cells can feasibly be the correct explanation of the non-occurrence of Gm determinants in the products of these cells.

Genes Gm^b and Gm^f are located very close to one another on the chromosome, so close that they are rarely, if ever, separated in meiosis in the Caucasian population studied (see Table I and below p. 1184). It is therefore unlikely that 1, but not the other, of these 2 genes should invariably be missing in myeloma cells owing to chromosomal loss in the (ontogenetic) history of the cell. These considerations favour the view that different Gm genes are present in the cell and can be switched on separately.

Using immunofluorescence microscopy Colberg and Dray (19) determined the genetic type of γ-globulin in rabbit plasma cells and concluded that individual cells contain the products of both of 2 allelic genes. If this conclusion is taken for granted it appears as if normal plasma cells may differ from myeloma cells in this respect. It is possible that Gm genes are inactivated to a varying degree in normal plasma cells; the myeloma cells may represent an extreme. The data on anti-Rh (see also references 5, 14, 20, 21) and other antibodies (9, 15, 16) suggest that in antibody producing cells clones in humans (5, 9, 14, 20, 21) and in rabbits (15, 16) certain Gm genes may be largely inactive or, possibly, involved in the synthesis of γ-globulin lacking the particular antibody specificity.

Plasma Cell Myeloma, a Single Cell Clone Committed to the Synthesis of 1 Type of γ-Globulin Molecules.—That no myeloma globulins contained the product of more than 1 of the genes, Gm^a, Gm^b, and Gm^f, gives strong support to the view that, as a rule, the myeloma cells derive from a single stem cell. The γ-globulin–synthesizing "machinery" of this stem cell is, or becomes, "locked" in such a way that its daughter cells can utilize only 1 of its Gm genes in the production of γ-globulin.

The concept of cell clones committed to the synthesis of one kind of γ-globulin,—one kind of antibody,—is well established (see reference 22). The plasma cell population of an individual appears to be heterogeneous also in respect of Gm type.

One may tentatively postulate that when a cell becomes committed to use 1 Gm (and 1 Inv) gene for its γ-globulin synthesis, this reflects a commitment to the production of 1 kind of antibody.

Anti-Rh Antibodies and Their Properties as Components in the Gm Test Systems.—It was early recognized that if an anti-Rh serum is to be used for Gm

coating it must be derived from an individual positive for the Gm factor in question (20, 21). On the other hand, high-titred incomplete anti-Rh sera from Gm(a+) or (b+) individuals were often found not to sensitize Rh-positive red cells to anti-Gm(a) respectively anti-Gm(b) (20, 21). After studies of myeloma globulins had shown that Gm(a) and Gm(b) are probably contained in separate molecules (4), it was realized that the inability of an anti-Rh serum to coat for, say, anti-Gm(a) is due simply to absence of Gm(a+) anti-Rh antibodies, even though the whole serum be Gm(a+) (5). Yet analysis of anti-Rh sera and antibodies with respect to Gm(a) and (b) could not always explain their properties as Gm coats; *e.g.*, why anti-Rh sera from Gm^b homozygotes often fail to coat for anti-Gm(b) (14).

The only 2 antibodies from individuals possessing the gene Gm^f that were Gm(f−) proved to be Gm(b+) (199 and M. B., see Table V). As a matter of fact, these two highly selected anti-Rh sera are more useful as Gm(b) coats than any of the others. We can now reasonably explain why often Gm(b+) anti-Rh sera do not coat for anti-Gm(b) or do it very poorly, no matter how much serum is used to coat a certain amount of red cells. Most of the antibody molecules are Gm(b−f+), and they block most Rh sites and thereby prevent attachment of further Gm(b+) antibody molecules. The observations on anti-Rh sera 199 and M. B. suggest that, to be a first quality Gm(b) coat, the anti-Rh antibodies of a serum should be Gm(a−f−). Similarly, serum 696, which was selected because the anti-Rh antibodies in this high-titred serum appeared to be clearly Gm(a−b−), now proved superior as a Gm(f) coat.

It is well known that incomplete antibodies can block the attachment of anti-Rh agglutinins to red cells. Obviously, an excess of one species can also block the attachment of another species of incomplete anti-Rh antibodies.

Anti-Rh antibodies of different Gm types in a serum compete for the sites on the red cells. Therefore the anti-Rh antibodies should include products of only 1 Gm gene, Gm^a, Gm^b, or Gm^f, if the red cells are to be coated most effectively for the corresponding anti-Gm.

On the Relationship between Gm(a) and Gm(x).—Gm(x+) individuals are practically always Gm(a+); about half of all Gm(a+) North Europeans are Gm(x+) (23). The population data are compatible with the concept that Gm(a) and Gm(x) are made be closely linked genes or that they are both formed by a single gene, called Gm^{ax} (23).

All the Gm(x+) myeloma globulins were found to be Gm(a+) (Table IV). This finding indicates, that Gm(x) occurs in the same molecules as Gm(a) and therefore supports the hypothesis that Gm(a) and Gm(x) are both formed by one and the same gene, the gene Gm^{ax}. The existence of Gm(a+x+) myeloma globulins has also been reported previously (4, 6, 8).

The frequencies of the 2 genes Gm^a and Gm^{ax}, as calculated from population data, are approximately equal (23). The relative frequencies of these 2 genes

appear to be reflected in the frequencies of their products, as represented by the myeloma globulins. Of 11 Gm(a+) myeloma globulins, 5 were Gm(a+x+) and 6 Gm(a+x−) (Table IV). Nilsson (8) classified 6 out of 17 Gm(a+) myeloma globulins as Gm(x+).

Allelism and Linkage of Gm Genes.—Though the present investigation did not include studies of pedigrees it does justify some conclusions as to the mode of inheritance of Gm^f. In large family and population studies of Caucasians, performed by several authors, Gm^b appeared to be an alternate allele of Gm^a (1–3). These studies together with the observation (see Table I) that the characters Gm(b) and Gm(f) practically always occur together in Whites appear to justify the conclusions, *first*, that genes Gm^b and Gm^f are located very close to one another on the chromosome and, *second*, that the chromosome segment with Gm^b and Gm^f and the segment with the gene Gm^a are homologous. This implies *further* that Gm^a is closely linked with another, as yet unidentified, Gm gene.

It should be recollected that Gm^f, like Gm^a, is involved in the synthesis of a major fraction of the γ_2-globulin. This (as well as other data considered below) suggests that Gm^f, rather than Gm^b, is the true allele of Gm^a. Gm^b, the products of which are found in only a minor fraction of the γ_2-globulin, should consequently be the allele of the postulated unidentified gene(s) associated with Gm^a. This is in accord with the above conclusion (p. 1181) that unidentified Gm genes participate in the synthesis of only a minor proportion of the γ_2-globulin molecules in individuals of common Gm types.

It is well established that genes Gm^a and Gm^b cannot be alleles in Negroes, as practically all Negroes are Gm(a+b+) (24). Of 10 American Negro sera available 7 were found to be Gm(a+b+f−), a very rare type in Caucasians, and 3 were Gm (a+b+f+). It is obvious that type Gm(f−) is much more common in Negroes than in Whites (see Table I). It has been estimated that around ⅛ of the gene pool of the American Negro population is of Caucasian origin (25). The occurrence of type Gm (f+) in 3 out of 10 American Negroes can be accounted for by Gm^f genes of Caucasian origin. However, Gold *et al* have found that type Gm(f^+) does, indeed, occur with a *low* frequency in African Negroes (10).

The gene Gm^a has a very high frequency in Negroes (2) and a much lower in Caucasians. Therefore a true allele of Gm^a has to be relatively infrequent in Negroes. Gm^f, but not Gm^b, fulfils this criterion. On the other hand, an observation that 25, out of 27 Chinese were Gm(b+f+) (10),—together with previous reports on the frequency of type Gm(a+) (2),—appears to indicate that the great majority of individuals in this population are Gm(a+b+f+); this would obviously preclude (at least for certain populations) the hypothesis that the specificities Gm(a) and Gm(f), or Gm(a) and Gm(b), are elaborated by genes behaving as alternate alleles.

Harboe *et al.* (6) found all of 6 myeloma globulins from Negroes to be Gm (a+b−) and not Gm(a+b+) as might have been suspected from the current hypothesis that all Negroes are homozygous for a gene Gm^{ab} (24). This finding was suggestive evidence, first, that Gm^a and Gm^b are indeed distinct, though closely linked genes in Negroes and, second, that Gm^a in Negroes, like in Whites, expresses its information in a much larger proportion of the γ_2-globulin molecules than Gm^b. It appears that in Negroes a gene complex with Gm^aGm^b predominates, whereas a complex with Gm^fGm^b is most common in Caucasians.

Grey and Kunkel have demonstrated the existence of 3 distinct classes of γ_2-myeloma globulins, each possessing characteristic antigenic determinants ascribable to the H (heavy polypeptide) chains (26). It has been shown that the Gm(a+) and the Gm(f+) myeloma globulins belong in 1, and the Gm(b+) in another, of these 3 antigenic classes (27). This is another strong argument against the hypothesis that Gm^b is the allele of Gm^a.

Neel (28) and others discussed the hemoglobin genes as a "remarkable example of the clustering of related genetic functions on a single mammalian chromosome." Apparently, the same statement can be made about the γ_2-globulin genes. There are 2 (or more) closely linked loci for Gm genes. It seems likely that there are 2 or more loci also for Inv genes.

The Gm genes are apparently engaged in the synthesis of the A (H) chains of the γ_2-globulin, the Inv genes in the synthesis of B (L) chains (29–31). Inv(a) and Inv(l) are characters which, like Gm(b) and Gm(f), generally occur together in Caucasians (32). More than 99 per cent of Europeans are either Inv(a+l+) or Inv(a−l−) (32). The current hypothesis has been that Inv(a) and Inv(l) are formed by one and the same gene, Inv^{al} (32). The findings on the anti-Rh antibodies of three Inv(a+l+) sera (see Results) suggest that Inv(a) and Inv(l) occur in separate molecules and therefore that they are formed by different genes. On this line of thought the relationship between genes Inv^a and Inv^l is analogous to that between Gm^b and Gm^f.

It may be remarked that if there exist 2 to 4 loci for A chain genes as well as for B chain genes at least as many as $(4 \times 4 =)$ 16, $(6 \times 6 =)$ 36, or $(8 \times 8 =)$ 64 types of molecules could possibly be formed in one individual, if the product of any of the A chain genes can be combined with the product of any of the B chain genes.

The existence of more than 1 locus for genes involved in the synthesis of polypeptide chains either of which can occupy a given position in the γ-globulin molecule considerably increases the number of genotypically determined distinct subtypes of the immunoglobulins. This reduces the amount of variation in the structure of these proteins that may have to be accounted for by agents other than inheritable genes.

SUMMARY

Gm types of sera from Caucasians and Negroes, of myeloma globulins, and of specific antibodies were investigated. In particular the relationship between the recently identified gene Gm^f and other Gm genes was analyzed.

1. Caucasians were, with rare exceptions, either Gm(b+f+) or Gm(b−f−).

2. In Negroes, on the other hand, type Gm(b+f−) was found to be common.

3. No myeloma globulins appeared to contain the product of more than 1 of the 3 genes Gm^a, Gm^b, and Gm^f, even though the large majority of normal individuals, as well as myeloma patients, possess 2 or all 3 of these genes.

4. The types Gm(*a*+b−f−) and Gm(a−b−*f*+) represented the 2 major groups among the γ_2-myeloma globulins; each comprised roughly 40 per cent. The types Gm(a−b+f−) and Gm(a−b−f−) are minor groups.

5. All Gm(x+) myeloma globulins were also Gm(a+). Approximately half of the Gm(a+) myeloma globulins were Gm(x+).

6. In the majority, but *not* in all, of Gm(a+) sera the anti-Rh antibodies were clearly Gm(a+). Similarly, the anti-Rh antibodies of Gm(f+) sera were most often, but *not* always, also Gm(f+). In contrast, only a minor proportion of Gm(b+) anti-Rh sera sensitized red cells to anti-Gm(b).

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