DISTRIBUTION AMONG THE γ-GLOBULIN MOLECULES OF DIFFERENT GENETICALLY DETERMINED ANTIGENIC SPECIFICITIES IN THE GM SYSTEM

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In Caucasians the 2 γ-globulin genes Gm^b and Gm^f are, as a rule, inherited together and form part of a gene complex that appears to be allelic to another complex with the gene Gm^a (1–3). No Caucasian myeloma globulins have been found to contain the product of more than 1 of the 3 genes Gm^a, Gm^b, and Gm^f (2, 4). Precipitating antisera can be used to distinguish 4 classes of γG-immunoglobulins with characteristic antigenic determinants ascribable to the H(heavy) polypeptide chains (5–7). It is postulated that each class corresponds to 1 H chain gene locus (4); that Gm^a and Gm^f occur at 1 of these, called the *We* locus after its antigenic type, and that Gm^b occurs at the closely linked *Vi* locus (1, 2, 4).

Reagents that give the same results as the original anti-Gm(b) of Harboe (8) with almost all Caucasian sera often give discordant results with sera from other races (9–11). In other words, non-Caucasians are often typed as positive with some, but not with other, "anti-Gm(b)" reagents. In a study of Japanese sera Ropartz *et al.* (9) found the anti-Gm(b) reagents to produce 3 different reaction patterns and named the hereditary factors detected, Gm(b^α), (b^β), and (b^γ). Steinberg and Goldblum (10) studied populations of different races and used the designations Gm(b^1), (b^2), (b^3), and (b^4).

The various Gm(b) (9, 10) and Gm(f) (1–3) determinants are all inherited together in Caucasians. Investigations of myeloma and anti-Rh proteins (2, 4) suggest that Gm(f) does not occur in the same γ-globulin molecules as certain Gm(b) determinants identical or closely related to Gm(b^γ) and (b^1) (11). However it is not known whether the other Gm(b) factors (*e.g.*, b^α, b^β, b^2, b^3, and b^4) are located on the same γ-globulin molecules as Gm(b^1) or Gm(f). The purpose of this study was to obtain information on the distribution among the γ-globulin molecules, primarily through the use of myeloma proteins, of the determinants identified by 18 anti-Gm(b) and anti-Gm(f) reagents.

Materials and Methods

Reagents.—The anti-Gm sera used gave test systems that were inhibited by Gm(+) sera diluted 1/100 or more, but not by Gm(−) sera even undiluted (12). The origins and designa-

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tions of the reagents are given in Table I. The reagents included: anti-Gm (b^7), (b^7), and (b^7) (9); the anti-Gm (b^2), (b^3), and (b^4) (10); and 5 anti-Gm (f) reagents (Table I).

Myeloma Globulins.—31 γG-myeloma globulins which had been typed for Gm(a), (b), and (f) (2, 4) were selected to comprise as many representatives as possible of the rare Gm(b+) type and of the less frequent H chain classes. All 31 were from American and Swedish patients;

TABLE I

Gm Test Systems and Anti-Gm Reagents Used

	Anti-Gm	Anti-Rh
Anti-Gm(a)		
1. B.A.		602
2. Stang.		—
Anti-Gm(f)		
3. Mau.[c]		Roehm.[h]
4. A.J.		Tö.[b]
5. Lu.[b]		Tö.
6. Fi.[b]		Tö.
7. 533802[d]		Tö.
8. Davis[h],	anti-b^2	Roehm.
Anti-Gm(b)		
9. A.B.[e]		—
10. I.G.	anti-b^7	2127[f]
11. All.[g]	anti-b^7	2127
12. 2247[f]	anti-b^7	—
13. Caz.[g]		2127
14. Gar.		2127
15. Th.[h]	anti-b^3	Vai.
16a. Bu.[h],	anti-b^4	Vai.
16b. Bu.[h],	anti-b^4	2127
17. 2357[f],	anti-b^3	Vai.
18. Let.[g],	anti-b^α	Vai.
19. 114[a]		2127
20. 2277[f],	anti-b^α	—

The sources of the anti-Gm reagents are indicated by the superscripts. [a] H. Fudenberg, San Francisco, California, [b] W. Göhler, Leipzig, Germany, [c] E. R. Gold, Bristol, England, [d] K. L. Goldsmith, London, England, [e] M. Harboe, Oslo, Norway, [f] Erna van Loghem, Amsterdam, Netherlands, [g] C. Ropartz, Rouen, France, [h] A. G. Steinberg, Cleveland, Ohio.

2 of them, *viz.* Hu. and La., were Negroes. The Gm(a+b−f−) and Gm(a−b−f+) myeloma globulins belonged to the major H chain class, the We class; the Gm(a−b+f−) myeloma globulins, to the Vi class; and the Gm(a−b−f−) myeloma globulins, to the Vi, the Ge, or the Ne class (4). Only myeloma sera containing at least 30 mg/ml of myeloma globulin and decreased amounts of normal γ-globulin (probably always below 5 mg/ml) and myeloma globulin preparations isolated as in previous studies (13, 14) were investigated.

In addition, 1 myeloma serum from a Chinese patient was available. The myeloma glob-

ulin was of γG-type by immunoelectrophoretic criteria. The γ-globulin concentration was 45 mg/ml as estimated by paper electrophoresis. The staining of the γ-region of the electrophoresis strip, outside the sharp γ_2-myeloma band, was much fainter than the corresponding region of normal serum strips. Thus, the serum contained about 40 mg/ml myeloma globulin and considerably less normal γ-globulin than sera from healthy persons.

Anti-Rh Antibodies.—20 anti-Rh sera from European and 2 from American donors were selected. Only sera found to make Rh+ red cells strongly agglutinable by 1 or another anti-Gm were included. The series does not reflect the frequencies of the different Gm types among random anti-Rh coats (*cf.* reference 2); in particular, Gm(b+) antibodies are intentionally over-represented.

Normal Sera.—73 Gm(a+) sera, to be typed with the various Gm(f) reagents, were from normal Swedish blood donors.

Gm Determinations.—Gm determinations of sera and other protein solutions are made with an agglutination-inhibition test. Anti-Gm sera agglutinate red cells coated with Gm(+) anti-Rh antibodies. 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. The techniques used for Gm typing have been described previously (2).

The myeloma sera and myeloma globulin preparations were tested in series of twofold dilutions to determine the inhibitory titers in the different Gm typing systems. The myeloma sera and preparations examined contained at least about 6 times as much myeloma globulin as normal γ-globulin (see above). 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 (*cf.* reference 2). This distinction was further facilitated by the fact that a myeloma globulin Gm(+) in respect of a particular Gm factor has a higher activity per unit protein than the total normal γ-globulin of a Gm(+) serum (*cf.* reference 2). The procedure used for Gm typing of myeloma globulins, which was described in greater detail in reference 2, is exemplified by the investigation of the Chinese myeloma serum (see Results).

The normal and the anti-Rh sera were typed in only 1 dilution, 1/10, in order to economize valuable reagents. The Gm types of *anti-Rh sera* were determined after absorption of the anti-Rh antibodies; 0.5 cc of the 1/10 dilution of the serum was absorbed twice with 0.2 cc of packed Rh+ red cells at 37°C for 60 minutes.

The Gm types of incomplete *anti-Rh antibodies* were determined by direct agglutination (Table IV). The antibodies were coated on Rh+ red cells and the coated cells tested with the anti-Gm agglutinators. Agglutination is taken to mean that the anti-Rh antibodies carry the Gm determinant in question; non-agglutination, that none, or only relatively few, of the antibodies carry the determinant. For example (see Table IV), the whole γ-globulin of serum I.M. is Gm(a+b+f+), whereas the anti-Rh antibody protein is, at least largely, Gm(a+b−f−). In the experiment of Table IV one volume of packed O Rh+ red cells was coated with 10 volumes of each anti-Rh serum diluted 1/5, or less, for 90 minutes at 37°C. The agglutinators used to test the coated cells were diluted according to their titers.

RESULTS

Myeloma Globulins.—Table II gives the Gm types of the Caucasian and the 2 Negro myeloma globulins. The Gm(b) specificities, except b^2 but including b^α, b^β, b^γ, b^3, and b^4 (some of which may be identical), were found together in the same Caucasian Vi type myeloma globulins. Gm(b^2) and the determinants

TABLE II

Gm Types of Selected Myeloma Globulin

Myeloma globulins		Gm test systems*																
H chain antigenic types	Designation	Gm(a)	Gm(f)					Gm(b²)	Gm (b)									
		1	3	4	5	6	7	8	10	11	13	14	15	16a	16b	17	18	19
We	G.S.	+	−	−	−	−	−	−	−	−	−		−				−	−
(γ_{2b})‖	O.L.	+	−	−	−	−	−	−	−	−	−		−				−	−
	E.S.	+	−	−	−	−	−	−	−	−	−		−				−	−
	E.H.	+	−	−	−	−	−	−	−	−	−		−				−	−
	J.A.	−	+	+	−	+	+	+	−	−	−		−				−	−
	E.P.	−	+	+	+	+	+	+	−	−	−		−				−	−
	M.P.	−	+	+	+	+	+	+	−	−	−		−				−	−
	789	−	+	+	+	+	+	+	−	−	−		−				−	−
	818	−	+	+	+	+	+	+	−	−	−		−				−	−
	844	−	+	+	+	+	+	+	−	−	−		−				−	−
	904	−	+	−	+	+	+	+	−	−	−		−				−	−
	Me	−	+	−	+	−	+		−				−					
Vi	S.H.	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
(γ_{2c})	K.B.	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
	276	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
	619	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
	224	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	
	589	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	
	Ap.	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
	Fe.	−	−	−	−	−		−	+				+					
	Man.	−	−	−			−		+			+	+	+	+	+	+	
	Hu. (Negro)§	−	−	−			−		+	+	+	+	−	+	+	+		
	Zu.:H‡	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
	Sh.	−	−	−	−	−	−		−				−					
	Vi.	−	−	−	−	−	−	−	−	−	−	−	−			−		
Ge	Ge.	−	−	−	−	−		−	−				−					
(γ_{2d})	Ro.	−	−	−	−	−		−	−				−					
	La. (Negro)	−	−	−	−	−		−	−				−					
Ne	Ne.	−	−	−	−	−		−	−				−					
(γ_{2a})	Ka.	−	−	−	−	−		−	−				−					
	Bl.	−	−	−	−	−		−	−				−					
	Mag.	−	−	−	−	−		−	−				−					
	Da.	−	−		−				−				−					

* The test systems used are indicated by numbers in the head of the Table. The numbers refer to Table I.

‡ Zu.:H is the paraprotein of a patient with Franklin's disease (15).

§ The Negro myeloma globulin Hu. was typed clearly negative with anti-Gm(b) Th. and with two other anti-Gm(b) reagents (not included in the Table); it was clearly positive with the other anti-Gm(b) reagents.

‖ Nomenclature of Terry and Fahey (6).

identified by 5 anti-Gm(f) reagents were as a rule found together in other myeloma globulins.

A few myeloma globulins were exceptional and reacted with some but not with other anti-Gm(f) or anti-Gm(b) reagents. Myeloma globulins J.A. and 904 were both negative with 1, but not with the same, anti-Gm(f); Me. was negative with 2 of the anti-Gm(f) reagents. Myeloma globulin Hu., from a Negro patient, was clearly negative with anti-Gm(b^3) Th. but clearly positive with another anti-Gm(b), identical or related to (*cf.* 11) anti-Gm(b^1). It is noteworthy that among the essentially Gm(f+) myeloma globulins as many as 3 lacked the antigenic determinant identified by 1 or another anti-Gm(f), whereas only the Negro myeloma globulin gave discordant results with different anti-Gm(b) reagents, despite the fact that the experiments on Gm(b+) myeloma globulins were more extensive than those on Gm(f+) myeloma globulins.

The paraprotein of the patient Zu. with Franklin's disease was included in Table II. This protein probably corresponds to Fc (fast) γ-globulin papain fragments (15). It carried all the Gm(b) specificities, except b^2 but including b^α, b^β, b^γ, b^3, b^4, and others, which suggests that they all occur in or close to the Fc part of the γG-molecule,

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 (*cf.* reference 2): (*a*) The inhibitory activity of Gm(b+) myeloma globulins was 4 to 32 times as high as that of the whole γ-globulin in Gm(b+) sera from normals; this difference in activity per unit protein between Gm(b+) normal and myeloma γ-globulins was found in all 10 Gm(b) test systems used. (*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-globulin in Gm(f+) normal sera.

The investigation of the Chinese myeloma serum will be described in greater detail since the results were essentially different from those obtained with any Caucasian myeloma serum. The following paragraph also illustrates how the Gm types of the myeloma globulins were determined (*cf.* reference 2).

Table III gives the results of the Gm determinations of the Chinese myeloma serum. Several Caucasian, 3 Japanese, and 3 Eskimo sera of type Gm(a+b+f+) were tested with the Gm(a), (b), and (f) test systems, and they gave closely similar inhibitory titers. (No normal Chinese sera were available.) The Chinese myeloma serum, on the other hand, was 8 to 16 times more strongly inhibitory in the Gm(a) and (f) systems, but less inhibitory in the Gm(b) systems, than the normal sera (Table III). The values for the Gm(a) and the Gm(f) activities (Table III) can hardly be accounted for by the normal γ-globulin; they must be ascribed to the myeloma globulin. Since the serum contained about 40 mg/ml of myeloma globulin, the Gm(a) and Gm(f) activities (per unit protein) of the myeloma globulin were about 2 to 4 times higher than the specific activities of the *total* γ-globulin of normal Gm(a+) and Gm(f+) sera. On the other hand, the Gm(b) (Gm(b^1) and Gm(b^2), *cf.* reference 11) titers can be ascribed to the residual normal γ-globulin.

Accordingly, the γG-myeloma globulin of the Chinese serum was grouped as Gm(a+f+b−), a type that has not been found among Caucasian myeloma globulins (2, 4, and Table II).

In view of the importance of this myeloma protein detailed investigation of its characteristics and isolation were carried out. Electrophoresis experiments in various media indicated a very homogeneous myeloma peak of slow mobility. Both the Gm(a) and Gm(f) characters were found associated with this peak utilizing totally different reagents than those used for Table III. The myeloma protein was of the We group with lambda type light chains.

TABLE III

Gm Characteristics of 1 Chinese Serum Containing about 40 mg/ml of a γG-Myeloma Protein

Test systems (see Table I)		Specific activity of the myeloma serum*
Gm(a):	No. 1	16
	No. 2	16
Gm(f):	No. 4	8
	No. 5	4–8
	No. 6	8
Gm(b):	No. 10	½
	No. 15	½

* The average activity of normal Gm(+) reference sera has arbitrarily, been given the value 1. For example; a normal Gm(a+) serum as a rule has a Gm(a) activity close to 1 (<2 but > ½). The Gm(a) activity of the Chinese myeloma serum was 16, *i.e.*, the inhibitory titre was 16 times higher than the average titre of normal Gm(*a*+) sera. In the two Gm(*b*) test systems, on the other hand, the myeloma serum had lower inhibitory titre than normal Gm(b+) sera.

Anti-Rh Antibodies.—Table IV gives the Gm types of anti-Rh antibodies. It is well established that even if the individual possesses a particular genetic determinant it may be absent from a specific antibody in his serum (2, 16, and others; see Table IV for many examples). The total γ-globulin comprises an extremely large number of different molecules whereas a specific antibody consists of a limited, sometimes a small, number of molecular species. The experiments of Table IV are intended to test a hypothesis based on the myeloma data above: the Gm(f) and (b^2) determinants are found together in certain molecules and the rest of the Gm(b) determinants are found together in other molecules.

We find, *firstly*, that anti-Rh antibodies reacting with 1 anti-Gm(f) generally reacted with the others (Table IV). Anti-Rh I.K. is exceptional as it was strongly agglutinated by anti-Gm(f) No. 3, but not by No. 4, 6, or 7. Anti-Gm(b^2) Davis agglutinated anti-Rh Roehm.

TABLE IV

Gm Types of Selected Anti-Rh Antibodies

Anti-Rh sera				Anti-Gm reagents*																		
Designation	Gm type of the serum‡			Gm(a)		Gm(f)				Gm(b^3)	Gm(b)											
	a	b	f	1	2	3	4	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Gar.	+	−	−	++	++	−	−	−	−			−		−		−	−	−	−			−
115	+	−	−	++	++	−	−	−	−			−		−		−	−	−	−			−
M.S.	+	±§	−	++	++	−	−	−	−			−		−		−	+	−	−			−
I.M.	+	+	+	++	++	−	−	−	−			−		−		−	−	−	−			
64	+	+	+	++	++	−	−	−	−			−		−		−	−	−	−			−
602	+	+	+	++	++	+	+	−	−			−		−		−	−	−	−			−
S.B.	+	+	+	++	++	+	+	−	−			−		−		−	+	−	−			−
89	+	+	+	++	+	++	++	++	++	−	−	−	−	−	−	−	−	−	−	−		−
Reb.	+	+	+	++	++	+	++	+	+	(−)	−	−	−	−	−	−	+	+	+	−		−
108	+	+	+	−	−	++	++	+	+			−		−		−	+	−	−			−
696	−	+	+	−	−	++	++	++	+			−		−		−	−	−	−			−
Tö.	−	+	+	−	−	++	++	++	++	+		−		−		−	(+)	−	−			−
Roehm.	−	+	+	−	−	++	++	++	++	++	(+)‖	+‖	−	−	−	−	−	−	−	−		−
Ham.	+	+	+	++	++	+	++	+	+			+		(+)		−	++	++	+			(−)
2238	−	+	+	−	−	++	++	+	++			+		+		+	++	++	++			+
Nic.				−	−		++	++	++			++		++		++	++	++	++	++	++	++
2126	−	+	+	−	−	++	++	+	+	(+)	++	++	++	++	++	+	++	++	++	++		+
I.K.	−	+	+	−	−	++	−	−	−			+		(+)		−	++	++	+			(−)
2086	−	+	+	−	−	−	−	−	−		++	+	+	(−)	−	(−)	++	++	++	(+)		(−)
2127	−	+	+	−	−	−	(+)	−	−	−	++	++	++	++	++	++	++	++	++	++	++	++
N.B.				−	−		−		−		++	+	+	(+)	(+)	(−)	++	++	++	(+)		−
Vai.	+	+	+	++	++	−	(−)	(−)	(−)		−	−	−	−	(−)	−	++	++	++	++	++	++

* The anti-Gm reagents used (to test the cells coated with the various anti-Rh antibodies) are indicated by numbers in the head of the Table; the numbers refer to Table I. The signs ++, +, (+), and (−) denote agglutination of different strengths and the sign − denotes no agglutination. An empty space indicates that no determination was done.

‡ The Gm types of the anti-Rh *sera* were determined (after absorption of the anti-Rh antibodies) with the test systems: Gm(a) No. 1, Gm(f) No. 4 and 7 (which gave identical results), Gm(b) No. 10 and 15 (which gave identical results on all the sera except M.S.).

§ Serum M.S. belongs to a Gm(b) type that is rare in Caucasians. It was positive with the Gm(b) test system 15 and negative with system 10 (Table I). A genetic study of the family is described in reference 11.

‖ The combination of anti-Gm(b) I.G. with anti-Rh Roehm. did not give a Gm(b) specific test system. Therefore the weak agglutination in this particular case may not be due to the anti-Gm(b).

strongly only when used undiluted. A higher concentration of anti-Gm(b^2) antibodies might have given strong agglutination also of other anti-Rh coats. But the reactions that could be obtained places agglutinator Davis together with the anti-Gm(f) rather than the anti-Gm(b) reagents.

Secondly, we find that anti-Rh antibodies reacting with 1 anti-Gm(b) as a rule reacted with all the other anti-Gm(b) reagents (Table IV). There are a few exceptions to this rule. Some of them are only apparent: the weakest agglutinators, No. 13, 14, and 20, do not give visible reactions with anti-Rh coats that react only weakly with stronger anti-Gm(b) agglutinators. This does not signify absence of some determinants; it is the finding to be expected when only a relatively small proportion of the anti-Rh antibodies are Gm(b+) (*cf.* reference 2). The anti-Rh antibodies of serum Vai. constitute a true exception to the rule: the anti-Gm(b) reagents 15 through 20, including anti-Gm(b^α), (b^3), and (b^4), agglutinated with maximal strength whereas the other anti-Gm(b) reagents were non-reactive. The Vai. anti-Rh protein essentially lacks the part of Gm(b) which is identified by Harboe's original anti-Gm(b), by anti-Gm(b^γ), and by anti-Gm(b^1) (*cf.* reference 11).

The results on anti-Rh antibodies given in Table IV may be summarized as follows: the anti-Rh antibody protein of Gm(b+f+) sera was in several instances Gm(b+f−) (2086, 2127, N.B., and Vai.) or Gm(b−f+) (89, Reb. 108, 696, Tö., and Roehm.). As a rule the various anti-Gm(b) reagents (except anti-b^2) reacted with the same antibodies and the same was true for the anti-Gm(f) reagents. It should be remembered that this series of anti-Rh sera was selected so that Gm(b+f−) anti-Rh antibodies, in particular, were over-represented.

Normal Sera.—5 anti-Gm(f) reagents (test systems 3 through 7) gave identical results when used to type 73 Gm(a+) Swedish sera; 55 of the sera were positive and 18 were negative with all 5 reagents. This indicates that possible variants of the Gm^f gene that determine only some of these Gm(f) specificities must be rare (in the germ cell lines) of Northern Europeans.

DISCUSSION

In this and in previous (2, 4) investigations no Caucasian myeloma globulins were positive for more than 1 of the factors Gm(a), (b), and (f) (Table II); myeloma globulins are Gm(a+b−f−), Gm(a−b−f+), Gm(a−b+f−), or Gm(a−b−f−), although the total normal γ-globulin of most myeloma patients and healthy individuals is Gm(a+b+f+) or Gm(a−b+f+) (2, 4). These observations might be explained by the following hypotheses: there are 3 distinct genes Gm^a, Gm^b, and Gm^f; each gene determines its own polypeptide chain; individual γG-molecules contain only one of the 3 types of chains; only 1 Gm gene is active in a myeloma cell clone (2, 4). The anti-Gm(b) reagents used in the studies just referred to were closely related to the original anti-Gm(b) of Harboe (8), which probably corresponds to (*cf.* reference 11) the anti-Gm(b^γ) of Ropartz *et al.* (9) and the anti-Gm(b^1) of Steinberg and Goldblum (10). In the present investigation many other anti-Gm(b) reagents were included, *e.g.* anti-Gm(b^α), (b^β), (b^2), (b^3), and (b^4), which in non-Caucasians give results very different from Harboe's anti-Gm(b) (9–11).

The association between all the Gm(b) specificities, except (b^2), in the same Caucasian Vi type myeloma globulins (Table II) suggests that they are all attributes of the polypeptide chain determined by the gene Gm^b at the *Vi* locus. For analogous reasons the data suggest that the Gm(f) and the Gm(b^2) determinants are attributes of the polypeptide chain determined by the gene Gm^f at the *We* locus. Since many individuals in non-Caucasian populations lack some, but not other, Gm(b) specificities (9–11), there probably exist alleles at the *Vi* locus that are similar or identical with the ordinary Caucasian Gm^b gene in one segment but different in another (*cf.* reference 11).

All the Gm(b) specificities (except b^2) appear to depend on the Fc pieces of H polypeptide chains. This was initially demonstrated for Gm(b^1) by Harboe *et al.* (17) and by Franklin *et al.* (18). Steinberg and Polmar (19) found that (b^3), and (b^4) (but not b^2) are also recovered in the Fc fragments after papain digestion. All 10 anti-Gm(b) reagents of this study reacted with the paraprotein of a patient, Zu., with Franklin's disease (Table II).

The anti-Rh antibodies of a given serum, as a rule, reacted with all or none of the anti-Gm(b) (except anti-b^2), and with all or none of the anti-Gm(f) reagents (Table IV). These findings support the conclusions drawn on the basis of the myeloma data: the Gm(b) and (f) determinants, which are inherited together in Caucasians, are carried by 2 categories of γG-molecules. The 1 category contains a polypeptide chain determined by the Gm^b gene; the other category a chain determined by the closely linked Gm^f gene. When a major proportion of the anti-Rh antibodies of a serum belongs to 1 of the 2 types, then the antibodies will appear Gm(b+f−) or Gm(b−f+) (Table IV). In other anti-Rh sera the 2 types of antibodies occur in amounts of the same order of magnitude. Both will then occupy a significant proportion of the Rh sites of the red cells and the anti-Rh protein is typed Gm(b+f+) (Table IV).

The results both on myeloma globulins and anti-Rh antibodies strongly support the view that Gm(b^2), formerly called Gm(b^w) (20), and Gm(f) are closely related. Steinberg and Polmar (19) felt that Gm(b^2) and Gm(f) are identical and based this opinion on genetic population and papain digestion studies. Fudenberg has observed that the anti-Gm(b^2) and an anti-Gm(f) often typed the same myeloma globulins as positive (21). It appears that Gm(b^2) is not carried by the same molecules as the other Gm(b) specificities, and that it is not determined by a gene at the same locus as Gm(b). This, we think, should be reflected in the nomenclature. Therefore the designations Gm^f, for the gene, and Gm(f), for the antigenic characteristics, are retained here.

Exceptional myeloma globulins and anti-Rh proteins reacted with some but not with other anti-Gm(b) or anti-Gm(f) reagents. 2 explanations of this phenomenon will be considered: (*a*) The individual has inherited a gene partly different from the ordinary Gm^b or Gm^f. (*b*) The individual has inherited the

ordinary Gm^b or Gm^f gene. But the myeloma or anti-Rh forming cells have been modified during ontogenesis so that the protein lacks certain of the expected genetic determinants. For example, part of the gene may have been lost or altered (*cf.* 22).

The former explanation is applicable to the anti-Rh antibodies of serum Vai. (Table IV). They contain the product of Gm^{sb}, a gene that is probably partly identical with, and partly different from, the ordinary Caucasian Gm^b gene (11). The myeloma protein Hu. most likely contains the product of still another allele at the *Vi* locus; it lacked a Gm(b^3) determinant and represented the only Vi type protein from a Negro.

3 of the Gm(f+) myeloma globulins and apparently also the I.K. anti-Rh protein were negative with 1, 2, or 3 of the anti-Gm(f) reagents. It is possible that each of these 4 individuals has inherited a different variant of the Gm^f gene identified by our anti-Gm(f) reagents. However, the results on normal sera suggests that such variant Gm^f genes, if they exist, are rare in (the germ cell lines of) Northern Europeans. The possibility is not ruled out that the ordinary Gm^f gene or some other component of these myeloma and anti-Rh forming cells has been altered during ontogenesis. If so, the alteration affects the structure of that part of the γG-molecule that carries the antibody combining site; Gm(f) specificities are recovered in the Fab (Slow) papain fragments (23–25, 19).

One Chinese γG-myeloma globulin, the only one available, was found to be Gm(a+f+b−). This type has not been encountered among Caucasian myeloma globulins, all of which have been grouped positive for only 1 or for none of the factors Gm(a), (b), and (f) (2, 4, and Table II). The data available might be explained by the hypothesis that, *in Caucasians*, anti-Gm(a) and anti-Gm(f) identify 2 genes at the *We* locus, called Gm^b and Gm^f, and that Gm^f is generally coupled with the gene Gm^b at the *Vi* locus. On the other hand, in some Chinese populations about 90 per cent of the individuals are Gm(a+f+) (3, 10), which indicates that both factors can be determined by 1 gene or by 2 genes on the same chromosome (*cf.* reference 2). Gm(a) and Gm(f) specificities are found in different γ-globulin papain fragments; Gm(a) in Fc (Fast) (17–19, 24, 25) and Gm(f) in Fab (Slow) fragments (23–25, 19). This suggests that Gm(a) depends on an amino acid sequence in 1 half and Gm(f) on a sequence in the other half of the H polypeptide chains. Therefore the Gm(a) and Gm(f) antigenic specificities are probably not determined by homologous gene segments. On this view, a Gm^{af} gene could arise from Gm^a and Gm^f, for example, by intragenic crossing-over, and the Gm(a+f+b−) Chinese myeloma globulin of this study may contain a polypeptide chain determined by a Gm^{af} gene. Consideration should also be given to the possibility that the 2 portions of the H chain at which the Gm(a) and Gm(f) specificities are found might represent separate polypeptide chains. This possibility has been raised by a number of

investigators (15, 26) although all efforts to effect a separation have been unsuccessful.

Since the H polypeptide chains of the We and of the Vi classes of γG-immunoglobulin are antigenically closely related, the genes at the 2 loci must have many nucleotide sequences in common. Phylogenetically these 2 loci may have arisen through duplication and subsequent differentiation of 1 ancestral immunoglobulin H chain gene. Evidence in the mouse indicates close linkage between structural genes for γA- and γG-H chains (27). This suggests that additional loci determining H chains of immunoglobulins may be clustered in the same chromosomal region as the *We* and *Vi* loci. It is of interest to note that the partial homology between the genes at the various loci might lead to displaced synapsis and consequent unequal crossing-over. As a result chromosomes with supernumerary H chain loci, and others with missing loci, would arise. Nance (28) and Smithies (29) have suggested that the thalassemias and related disorders of hemoglobin production might be due to unequal crossing-overs involving the structurally similar β, γ, and δ loci. Similarly, deficient and duplicated complexes of H chain genes might account for aberrant patterns of immunoglobulin synthesis.

SUMMARY

Isolated myeloma proteins and anti-Rh antibodies were utilized for determination of the distribution among the γ-globulin molecules of various Gm(b) and Gm(f) determinants. All of these genetic factors are as a rule inherited together in Caucasians, but not in other races. The Gm(b) determinants, except (b^2), were found together in the same Caucasian myeloma globulins of the Vi H chain group, which never carried Gm(f) or (b^2). The Gm(f) and (b^2) determinants were found together in other myeloma globulins, of the We group. Anti-Rh antibody molecules appeared to be similar to myeloma molecules in these respects. A few myeloma proteins and anti-Rh antibodies were encountered which reacted with some but not other anti-Gm(b) or anti-Gm(f) reagents; the only available Vi type myeloma protein from a Negro, specifically lacked the Gm(b^3) factor.

The observations might be explained by the following hypothesis: In Caucasians with the common Gm^f Gm^b gene complex the Gm(b) antigenic determinants, except (b^2), co-occur in certain molecules, which contain a polypeptide chain determined by the Gm^b gene; the Gm(f) and (b^2) determinants co-occur in other molecules, which contain a polypeptide chain determined by the Gm^f gene. Individuals that lack some Gm(b) or Gm(f) determinants most likely have a gene partly different from Gm^b or Gm^f.

The value of studies with myeloma proteins from individuals of different racial groups was apparent from this study. One myeloma protein from a Chinese was unique in that it was both Gm(a+) and Gm(f+).

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