THE GENETIC CONTROL OF γ-GLOBULIN HEAVY CHAINS

STUDIES OF THE MAJOR HEAVY CHAIN SUBGROUP UTILIZING MULTIPLE GENETIC MARKERS*

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(Received for publication 18 January 1967)

Two independent gene regions are operative in the control of γG–globulin; the Gm and Inv loci (1). At the Gm locus, over 20 different genetically controlled antigens have been described to date, all localized to the heavy chain subunit of γG–globulin (1). Some of these factors are closely associated with one another, others segregate as alleles, and a few fit only partially into either classification (1). The multiplicity of these genetic antigens, with wide variations in different populations, has led to confusion in the interpretation of their interrelationships. The problem has been clarified considerably through the delineation of four different heavy chain subgroups of γG–globulin (2, 3), each associated with its own independent group of genetic antigens (4). The accumulated evidence indicates that the heavy chains of each subgroup are products of genes at separate but closely linked genetic loci (4).

The present studies were concerned with the genetic control of the major heavy chain subgroup, γG1.[1] The two most intensively studied factors of this class are Gm(a) and Gm(f). Although in most populations they are distributed as alleles (5), they present certain problems in that they appear to be localized to different parts of the heavy chain, i.e. Gm(a) is on the papain Fc fragment (6) while Gm(f) is on the Fd fragment (7); and they do not segregate as alleles in Mongoloid populations (8). Earlier studies from this laboratory have shown that monospecific typing reagents could be elicited by immunizing rabbits with isolated myeloma proteins or fragments thereof (9). This approach was utilized recently to delineate Gm(z) (10), a γG1–genetic antigen related to Gm(a). In the present work a second γG1–genetic factor, Gm(y), elicited by similar methods, was described. With the availability now of four genetic markers, the genetic control of γG1–heavy chains could be advantageously studied. These studies have resulted in the characterization of two major types of heavy chains in Caucasians controlled by allelic genes, as well as a further understanding of the unusual heavy chains found in Mongoloid peoples.

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* This investigation was supported by Public Health Service Grant No. Am AI 09792.

‡ United States Public Health Service Special Fellow F3 AM 20, 122-02.

[1] The nomenclature used for the heavy chain subgroups of γG–globulin are those recently adopted by a subcommittee of the World Health Organization: γG1(We or γ_{2b}); γG2(Ne or γ_{2a}); γG3(Vi or γ_{2c}); γG4(Ge or γ_{2d}).

Materials and Methods

Normal human sera from different races were furnished by Dr. F. H. Allen, Dr. H. Borel, Dr. R. Prendergast, Dr. A. Bearn, Dr. K. Berg, Dr. L. Martensson, and Dr. A. Kuramota. Myeloma proteins and macroglobulins were obtained from the serum of patients with multiple myeloma and macroglobulinemia, and were isolated by zone electrophoresis (11). Several myeloma proteins were obtained from Dr. K. Takastuki, Dr. E. van Loghem, and Dr. L. Martensson.

Papain digestion of gamma globulin and myeloma proteins was carried out for 16 hr under the conditions recommended by Porter (12). Pepsin digestion was performed at a protein

TABLE I

Reagents Used to Determine Gm Factors

Gm factor	Agglutinator	Reciprocal of dilution	Source	Anti-D	Reciprocal of dilution
(z)	MERS	30	Rabbit	AD_3	5
(a)	SMEJ	35	Human	19	5
	WE	30	Rabbit	19	5
(f)	ME	200	Rabbit	AD_3	5
	AJ	16	Human	1894	5
(y)	GR	5	Rabbit	AD_3	2
(x)	WEIS	4	Human	Jack	3
(20)	GARZA	300	Human	Nison	2
(b)*	FE	50	Rabbit	Had	5
	BA	100	Rabbit	Had	5
	R	5	Human	Had	5
(g)	VI	128	Baboon	4752	2

* Gm(b) agglutinators Fe and Ba detect Gm($b^{3,4}$) while R detects Gm(b^1).

enzyme ratio of 100 to 1 in 0.1 M acetate buffer, pH 4.1 (13). Heavy and light chains were isolated according to the methods described by Fleischman, Pain, and Porter (14). Reduction was carried out for 1 hr using 0.2 M 2-mercaptoethanol. Alkylation with 0.3 M iodoacetamide was followed by dialysis overnight against 1 M propionic acid. The chains were then separated on a 2.5 × 75 cm Sephadex G-100 column equilibrated in 1 M propionic acid (15).

The method employed in recombination studies was as follows (16): 1 ml of heavy chains at 1 mg/ml and ½ ml of light chains at the same concentration were mixed in a collodion bag and then dialyzed successively for 6–12 hr against 1 M propionic acid × 1, 0.1 M propionic acid × 1, 0.01 M Na acetate at pH 5 × 2, and 0.01 M Na acetate in Tris 0.0005 M at pH 7.6. A protein determination was made of the sample and it was tested in the appropriate typing system. The first dilution of the sample was made in equal parts of 0.3 M saline. The remaining dilutions were in isotonic saline. All protein determinations were by the method of Lowry et al. (17).

Genetic typing for Gm(a) and (b) using agglutinators SMEJ, WE, and FE was carried out by the slide method while the remainder of typing was performed by the tube method. These techniques have been described previously (6). The genetic agglutinators and the source from which they were obtained are listed in Table I. The anti-D coat used in each case was also noted. All of the rabbit source and the one baboon source anti-Gm agglutinators have been compared in the past to human source reagents and the typing specificity has always been similar (9). Gm(a) typing and Gm(b) typing in many cases was done with more than one typing reagent. Agglutinator WEIS and R were provided by Dr. H. Borel. Agglutinator MO and anti-D coat M used for the Inv(1) typing was obtained from Serum-Institut, Heidelberg, Germany. Agglutinator GARZA and anti-D coat Nison was supplied by Dr. H. Fudenberg. The specificity of Gm(b) agglutinators FE and BA was determined by Dr. A. Steinberg as anti-Gm($b^{1,3,4}$). Gm(g) typing was done by Dr. J. Natvig. The baboon antiserum used for typing Gm(g) was supplied by Dr. Moor-Jankowski and the Facility for Forensic Serology, New York City. A small number of sera were Gm(s) typed by Dr. L. Martensson. The World Health Organization notations for the genetic factors are as follows (18): Gm(a) is Gm(1); Gm($b^{1,3,4}$) are Gm(5, 13, 14); Gm(x) is Gm(2); Gm(f) is Gm(4); Gm(g) is Gm(21); Gm(z) is Gm(17); and Gm(y) is Gm(22).

Three adult rabbits were immunized as follows: two rabbits received a whole isolated Gm(a−f+) myeloma protein; and one rabbit received the isolated Fc fragment of a Gm(a−f+) myeloma protein. All injections were intramuscular after the proteins were emulsified in equal volumes of complete Freund's adjuvant. The initial immunization was 3–5 mg and booster injections of 2–3 mg of protein were given every 3 wk. The antisera were first studied after 10 wk of immunization.

For testing, each rabbit antiserum was divided into two aliquots, and each aliquot was absorbed with one-third of its volume of normal human Caucasian serum. One absorbent serum had the phenotype Gm(a+f−b−) and the other Gm(a−f+b+). The absorbed sera were permitted to remain at 4°C for 3 days, and then the supernatants were decanted and tested against a battery of 15 different anti-D coats on Rh + red blood cells. The agglutinating activity was read by eye in 10 × 75 mm test tubes after the coated cells and absorbed rabbit antiserum had been incubated at room temperature for 1 hr. Agglutination was graded from one to four +. In most cases the absorption removed all agglutinating activity against coated red cells. Any remaining agglutination was studied further, and it was considered particularly suggestive if the two different absorptions left the same antiserum with substantially different levels of agglutination. Next the rabbit antiserum was diluted to produce a two + agglutination and was then inhibited with panels of myeloma proteins possessing different heavy chain subgroups, and normal human sera of differing phenotypes. In this manner a rabbit antiserum was found which detected a new genetic antigen. This antiserum was from a rabbit immunized with an intact Gm(a−f+) myeloma protein. Genetic typing was carried out with this new genetic typing system using the same tube technique as employed for the known typing systems. The new genetic factor was tentatively designated as Gm(y) (19).[2]

RESULTS

Studies on Gm(y).—This new human genetic antigen was detected in sera and on isolated myeloma proteins of Caucasians and Negroes by means of rabbit antisera. Of 18 isolated γG1–myeloma proteins, Gm(y) was only present on the 11 which were Gm(a−f+), and on none of the 7 which were Gm(a+f−)

[2] Gm(y) has been designated Gm(22) by the World Health Organization nomenclature subcommittee.

(Table II). In sera also, Gm(y) was completely concordant with Gm(f). It was obvious that in Caucasians and Negroes Gm(y) and Gm(f) were closely related. They could be distinguished however by enzymatic separation of Fr. II, γ-globulin isolated from single positive sera, or Gm(z−a−f+y+) myeloma proteins. After papain treatment all of the Gm(y) activity could be found in the Fc fragment in as high an inhibitory capacity on a molar basis as in the in-

TABLE II

Phenotypes of Caucasian and Negro Sera and Myeloma Proteins

	Gm Factors				No. of sera tested	
	(z)	(a)	(f)	(y)	Caucasian	Negro
Sera	+	+	+	+	42	2*
	+	+	−	−	8	15
	−	−	+	+	90	0
Isolated myeloma proteins	+	+	−	−	5	2
	−	−	+	+	11	0

* This phenotype was unusual in the Negro race and may represent genetic admixture.

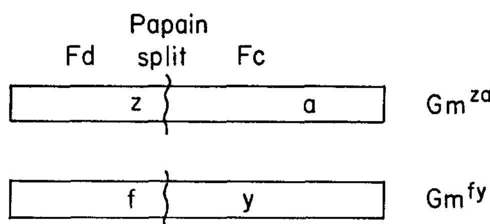

FIG. 1. Diagrammatic representation of the localization of Gm antigens on γG1-heavy chains. The two major types of heavy chains found in Caucasians are shown, with the positions on the Fd and Fc fragments of each pair of genetic antigens. The allelic genes controlling these chains are indicated on the right.

tact protein. Gm(f) was always present in the Fab fragment, as noted in earlier studies (7). With pepsin digestion, known to break up the Fc fragment, Gm(y) was completely lost while Gm(f) was unaffected, providing further evidence of the separate molecular localization of the two related factors. The data indicated that Gm(f) and the new antigen Gm(y) were present on the same γG1-immunoglobulin molecules but that they were localized to different fragments of the heavy chain.

Gm(y) and Gm(z) were studied on isolated myeloma proteins of the γG2-, γG3-, and γG4-subgroups, on five isolated monoclonal macroglobulins, and on seven isolated γA-myelona proteins. None of these proteins showed any degree of inhibition.

Pairs of Genetic Antigens on the γG1–Heavy Chains of Caucasians.—As reported earlier Gm(z) and Gm(a) are always concordant in Caucasian and Negro sera and myeloma proteins (10). The same was true for Gm(f) and Gm(y) as discussed above (Table II). These relationships strongly suggested that each pair of genetic antigens characterized one distinct variety of γG1–heavy chain as seen in the schematic representation in Fig. 1. The structural genes controlling each of these polymorphic varieties of heavy chain were designated as Gm^{za} and Gm^{fy} respectively. The distribution of these factors in

TABLE III

Gm Factors of Different Races

	Subgroup and Gm factor					Races					
	γG1				γG3	Cauc.‡	Negro	Indian‡	Japa-nese	Chinese	E. Isl.‡
	z	a	f	y	b*						
Sera	+	+	+	+	+	42	2	37	27	65	23
	+	+	−	−	±	0 8	7 8	4 45	70 102	3 9	0 13
	−	−	+	+	+	90	0	2	0	0	0
	−	+	+	+	+	0	0	1	0	107	12
Isolated γG1-myeloma proteins	+	+	−	−	−	5	2	0	3	1	0
	−	−	+	+	−	11	0	0	0	0	0
	−	+	+	+	−	0	0	0	2	3	0

* The typing system used detected Gm ($b^{3,4}$).

‡ The populations included were Cauc. = Caucasians: Indian = Asian Indians: and E. Isl. = Easter Islanders.

Caucasians indicated that the two genes segregated as alleles (Table II and III). The presence of either one or the other of the pairs on all isolated γG1–myeloma proteins of Caucasians substantiated the supposition that these associated factors shared the same molecules (20).

The Simultaneous Occurrence of Gm(f), (y), and (a) on Heavy Chains of Mongoloid Races.—The study of sera and myeloma proteins from Mongoloid peoples revealed a different distribution of the γG1–genetic antigens than found in Caucasians or Negroes (Table III). Five of the isolated Mongoloid myeloma proteins contained three genetic factors, Gm(f), (y), and (a). The inhibitory

capacity of the isolated myeloma proteins in all three typing systems was as high or higher than that of positive sera, indicating that this finding was not due to the presence of contaminating normal γ-globulin. These results suggested immediately that in Mongoloid persons Gm(a) and Gm(z) would have to be on different molecules, a fact substantiated by the presence of many Gm(z−a+f+y+) sera in such populations. This is in striking contrast to the situation for Caucasian and Negro sera where Gm(z) and (a) are always concordant. It was evident that there must be molecules with three genetic antigens, Gm(f), (y), and (a), on the same heavy chains and the gene coding for this heavy chain could be designated as Gm^{fya}. The population data suggested that the two genes controlling γG1-heavy chains in Mongoloid populations were Gm^{fya} and Gm^{za}. There was no evidence for the existence of the common Gm^{fy} gene in Mongoloid populations where all individuals are *Gm*(a+) (1).

Possible Mechanisms for the Development of the Gm^{fya} *Mongoloid Gene.*—The

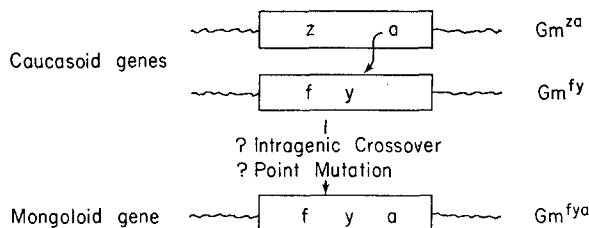

FIG. 2. Representation of the allelic Caucasoid γG1-genes and the unusual Mongoloid gene. Possible mechanisms for the development of the Mongoloid gene are indicated.

two Caucasoid γG1-heavy chains, Gm(f+y+) and Gm(z+a+), coded for by allelic genes, differed from the Mongoloid heavy chain Gm(f+y+a+) in the relationship between the genetic antigens of the Fc and Fd fragments. Gm(a) was no longer associated with Gm(z) but rather with the Fd marker from the second variety of γG1-heavy chain, namely Gm(f). Gm(y) and (a) were now both on the same fc fragment, the Gm(y) retaining its close linkage to the Gm(f) antigen. This information suggested that some alteration of genes, similar to the Caucasian types, might have resulted in the Mongoloid gene, Gm^{fya}, as outlined in the schema in Fig. 2. One possibility is that an intragenic crossover between the two allelic genes occurred. The reciprocal products of such a crossover would be the genes Gm^{fya} and Gm^{z}. There was no evidence for the existence of this latter gene in humans although this phenotype is present in certain nonhuman primates.[3] Any postulated changes in the genes, of course, could have occurred in the reverse direction. The above events could be explained equally as well by a point mutation on the Gm^{fy} Caucasian gene (see Fig. 2).

[3] Litwin, S. D. Unpublished observations.

When Gm(y) was found on the Fc fragment of all γG1-myeloma proteins which were Gm(a−), it was thought that Gm(y) would segregate as an allele of Gm(a). The distribution of these two genetic factors in Caucasian and Negro races was consistent with such allelism but in Mongoloids the incidence of both factors was too high. This discrepancy can be explained by the existence of the Mongoloid gene, Gm^{fya}.

Additional Studies Regarding the Origin of the Gm^{fya} Gene.—It was thought of interest to find out whether the Mongoloid gene, Gm^{fya}, could code for Gm(x), for this could potentially be of value in distinguishing between postulated genetic changes. Gm(x) is a genetic antigen found variably on Caucasian Gm(z+a+) heavy chains (21). If Gm^{x} were present on the Mongoloid gene, it would weigh in favor of a close relationship between Caucasian and Mongoloid genes and suggest a crossover, since the chance of a double point mutation

TABLE IV

Gm(x) and Gm(20) Typing of Mongoloid Sera*

Gm phenotype	Gm(x) No. of sera		Gm(20) No. of Sera	
	+	−	+	−
z a − −	3	6	34	9
z a f y	17	36	16	1
− a f y	0	75	8	5

* Only Chinese sera were typed for Gm(x), while both Japanese and Chinese sera were typed for Gm(20).

creating both Gm^{a} and Gm^{x} on the Gm^{fy} gene was small. This information could not be obtained by a study of isolated myeloma proteins since there were only five Mongoloid proteins of Gm(z−a+f+y+) phenotype available (Table III). All of these proteins were Gm(x−). Since the incidence of Gm(x) among Mongoloids ranges from 5 to 15% among the Chinese, and 30 to 45% among the Japanese (22), it was possible that the above myeloma proteins could have arisen by chance in Gm(x−) persons. To clarify this point, 137 Chinese sera were typed for Gm(x). The 75 sera which were Gm(z−a+f+y+) and presumed to be homozygous for Gm^{fya} were all Gm(x−). The remaining 62 sera, phenotypes Gm(z+a+f−y−) and Gm(z+a+f+y+), were considered to be carrying the gene Gm^{za} either as homozygotes or heterozygotes. They had an incidence of Gm(x) of over 30% (Table IV). It was concluded that Gm^{x} was not found on the Mongoloid gene Gm^{fya} but was present on the Mongoloid Gm^{za} gene. This evidence was consistent with either a crossover or a point mutation but failed to provide possible support for the former. Another recently described genetic antigen, Gm20, (23) that is present in most but not all Gm(a+) sera was studied in a similar fashion. In Table IV are shown 73 sera

that were typed for this factor. It may be readily seen that both the Gm^{za} and Gm^{fya} homozygotes could be either Gm20 positive or negative. This factor was variably associated with Gm(a) in both types of Mongoloid heavy chains.

Further Studies on the Localization of Gm(z).—Gm(z) represents a genetic antigen comparable in several respects to Gm(f) but present (in Caucasians) in a species of γG1-heavy chain controlled by an allelic gene. Both of these factors, Gm(f) and Gm(z), are present in the Fab fragment (the only two Fab Gm antigens to date) and both are influenced by separation of the subunit

TABLE V

Inhibition of Whole Proteins and Protein Fragments in Test System for Gm(z) and Gm(a)

	Lowest protein concentration for detection, mg/ml	
	Gm(z)	Gm(a)
Myeloma protein*		
Intact myeloma protein	0.015	0.06
Papain Fc	>1	0.10
Papain Fab	0.010	>1
Pepsin Fab	0.015	>1.2
Heavy chain	0.220	0.06
Light chain	>1.4	>1.4
Recombined chains	0.035	—
Fr. II γ-globulin		
Intact Fr. II	0.030	0.06
Papain Fc	>1	0.12
Papain Fab	0.030	>1
Pepsin Fab	0.015	>1.4
Heavy chain	0.280	0.12
Light chain	>1	>1
Recombined chains	0.045	—

* An isolated γG1-myeloma protein of phenotype Gm(z+a+f−y−).

chains. Studies on Gm(f) has shown it to be specific to the heavy chain but dependent on the heavy-light chain combination for its antigenicity (7). In order to determine more exactly the position of Gm(z), further investigations were carried out similar to those for Gm(f). The results can be seen in Table V for an isolated myeloma protein CO of phenotype Gm(z+a+f−y−) and human Fr. II γ-globulin. The Fc fragment was Gm(a+) and Gm(z−) whereas both papain or pepsin Fab fragments were Gm(z+) and Gm(a−). The respective fragments on which each factor was localized were approximately as inhibitory as was the intact myeloma protein or Fr. II. The isolated light chain

was always Gm(z−) while the heavy chain retained a small fraction of the inhibitory capacity of the intact protein. This last observation could conceivably be due to contaminating light chains in the heavy chain preparations but it should be noted that these results were the same using several different heavy chain preparations, some of which had little or no contaminating light chains by antigenic analysis. To determine which chain contained the Gm(z) specificity, recombination studies were conducted. Four sets of isolated heavy and light chains, two from Gm(z+) and two from Gm(z−) proteins, were used. In the first series of experiments four different combinations were arranged, all of which had heavy chains from a Gm(z+) protein but light chains of different types including both kappa and lambda type light chains from proteins which were Gm(z+) and (z−). The heavy chain from the Gm(z+) protein restored the Gm(z) antigen after recombination with all of these light chains. In a second group of experiments the same four recombinations were employed except that the heavy chain was from a Gm(z−) myeloma protein. In none of these latter experiments was the genetic factor restored. From these results it was concluded that Gm(z) was specific for the heavy chain (Fd) portion of the Fab fragment but that the factor was not fully expressed unless the light and heavy chains were combined. In view of the fact of the similarity of these data to those known for Gm(f), it appeared that Gm(f) and Gm(z) might occupy closely homologous positions on the heavy polypeptide chain.

Gene Complexes for the γG1–, γG2–, and γG3–Heavy Chain Loci.—The studies thus far presented have only concerned the genetic antigens of the γG1–subgroup of γ-globulin. Their delineation and study has also proved of considerable utility for understanding relationships to other subgroups of γ-globulin where genetic antigens are also available. It has become apparent that the genes controlling the γG2– and the γG3–heavy chains are very closely linked to those for γG1 (4, 25). However in different racial groups the gene complexes for these three subgroups are strikingly different. Table VI shows the major gene complexes observed. This is based in part on the very recent data on Gm(n) in the γG2–subgroup (25) and the studies of van Loghem and Martensson on the Gm(b) antigens of the γG3–subgroup (24). The Caucasian gene complexes contrast particularly with the Negroid types. The Gm^{za} gene of Caucasians is always associated with the Gm^{g} gene but for Negroids the Gm^{za} gene is always associated with one of the Gm^{b} genes and the close relationship in Caucasians of the Gm^{b} gene to the Gm^{fy} gene does not exist. Some type of cross-over mechanism seems almost certainly to be responsible for these different relationships. The fact that multiple markers are interchanged argues strongly against point mutation as an explanation for the differences.

The gene complex, which included the unusual Mongoloid gene, Gm^{fya}, was also of particular interest. It can be seen as the last vertical column under the Mongoloid population in Table VI. These results were obtained from studies of

selected sera homozygous for Gm^{fya}, of phenotype Gm(z−a+f+y+). All of the genetic factors present in these sera would be coded for by Gm^{fya} at the γG1-locus and by the linked genes at the other two loci. A large group of such Mongoloid sera were all Gm(b^1+, b^3+, b^4+) and Gm(n+) (25). It was therefore evident that Mongoloid γG1-gene, Gm^{fya}, was closely linked to the γG2-gene, Gm^n, and the γG3-gene, $Gm^{b^{1,3,4}}$ (footnote 4).

The second frequent Mongoloid γG1-gene, Gm^{za}, was evaluated in a similar manner. All of the Gm^{za} homozygous sera were Gm(n−) indicating that Gm^{za} was in all probability linked to the presumed allele of Gm^n, a genetic factor as yet not directly measurable. In Table VI this is shown for the gene complexes of the Mongoloid race by leaving the γG2-locus open. As evident in this table the

TABLE VI

Gene Complexes in Different Racial Groups Arranged in Vertical Fashion According to the Subgroup of Heavy Chain Involved

	Caucasoid				Negroid				Mongoloid			
		x									x	a
γG1	a	a	y	y	a	a	a	a	a	a	a	y
	z	z	f	f	z	z	z	z	z	z	z	f
γG3	g	g	b^0	b^0	b^0	b^0	b^0	b^0	b^0	g	g	b^0
			b^1	b^1	b^1	b^1	b^1	s	st			b^1
			b^4	b^4	b^4		b^4					b^4
			b^5	b^5	b^5	c^5	b^5	b^5	b^5			b^5
			b^3	b^3	b^3	c^3	b^3	b^3	b^3			b^3
γG2			n									n

situation is complicated at the γG3-locus where Gm^{za} can be associated with either Gm^g (27) or Gm^b. The latter linkage was first studied by Martensson (26) who showed that Mongoloids often had the genetic antigens, Gm(s, b^3) with (b^1) absent, always associated with Gm(a) but never with Gm(f) The present study with the additional information on Gm(z) is in agreement. A group of Gm^{za} homozygotes were Gm(b^3+) and (b^1−). 11 of this group that could be types for Gm(s) were all positive. This group would correspond to those depicted in the first vertical column under Mongoloids in Table VI.

DISCUSSION

The present studies indicate that the heavy chains of the γG1-subgroup in Caucasians are coded for by two allelic genes, Gm^{za} and Gm^{fy}. The protein

[4] The presence of Gm b^0 was determined from information in separate studies of the Gm(b) antigens. Dr. E. van Loghem has typed several of these Mongoloid sera homozygous for Gm^{fya} and found they were all positive.

product of each gene contains two genetic markers each on a different segment of the heavy chain. This was most apparent through studies of Caucasian myeloma proteins of the γG1–type. All of these proteins always contained either the pair of antigens (z) and (a) or the pair (f) and (y). The members of each pair were readily separated by papain splitting of the myeloma proteins into Fc and Fd fragments. Similar results were obtained with isolated normal γ–globulin, and genetic typing of a large number of Caucasian sera showed strict concordance between the members of each pair of antigens. The accumulated evidence indicated that the members of each pair occurred together on the same γ–globulin molecule, but at different sites. It seems probable that similar relationships will hold for the Gm(b) genetic antigens of the γG3–subgroup. Here at least four distinct antigens (1) have been delineated through studies of different racial groups, that in Gm(b) Caucasian sera occur together. However, these all occur on the Fc fragment and thus far have not been separated by enzymatic cleavage. Also two other genetic antigens, Gm(x) and Gm(20), occur only together with Gm(z+a+) γ–globulins (21, 23). Because their appearance is variable it is believed that they are the result of simple polymorphic variations in the Gm^{za} gene. The genetic antigen Gm(p) (28), which has proven difficult to study, is associated with Gm(f+) proteins (29) and may be closely related to Gm(y).

In Mongoloid populations a very different gene has become apparent, the Gm^{fya} gene. Previous studies (30) utilizing Gm(a) and (f) had indicated the unusual nature of this gene and this has been further clarified through the use of the additional marker Gm(y). Here the Gm(a) antigen occurs on the same proteins as the (f y) antigens which is never the case in other racial groups. In this case evidence is clearly available that each of the three antigens is in a different position on the same heavy chain. Thus, among γ–globulins, the occurrence of multiple genetic antigens on the same polypeptide chain is the general rule. It seems highly likely that each of these antigens results from independent amino acid substitutions at a separate site in the polypeptide chain. Two amino acid differences have been demonstrated for the Fc fragment of Gm(a+) versus Gm(a–) γ–globulins (31, 32). Studies of different genetic types of rabbit γ–globulins have always shown multiple peptide differences (33). The latter observation, initially difficult to understand, is in complete harmony with the results on the multiple genetic antigens of human γ-globulin.

There has been considerable speculation concerning the possibility that two separate structural genes govern the synthesis of the Fc and Fd fragments of the heavy chains as well as the variable and constant portion of the light chains (34). The present studies provide certain information on this point in that the genetic antigens on the Fc fragment and those on the Fd fragment containing Gm(z) and (f) are under the same genetic control. If Gm(f) and Gm(z) occur in the variable area of the Fd fragment the present results suggest that this area is a product of the same gene that codes for the Fc portion. Unfortunately,

however, the exact position of these markers in the Fd fragment is not known. It is possible that papain produces an artificial split of the heavy chains not exactly related to the variable versus constant portion of the chain. Thus some of the constant portion might appear in the Fd fragment and it might be in this area that Gm(f) and Gm(z) are found. Further studies with additional genetic markers in other areas of the chains as well as the determination of exact positions in the chains are required to answer this question conclusively.

The contrast between the allelic Mongoloid genes, Gm^{fya} and Gm^{za}, and the allelic Caucasian genes, Gm^{fy} and Gm^{za}, is of special interest. A number of possibilities concerning genetic events might be cited to explain these differences. An intragenic crossover between two genes similar to those in Caucasians could account for the development of the Gm^{fya} gene. Also a point mutation on the Caucasian Gm^{fy} gene perhaps could result in the Gm^{fya} gene. An attempt was made in the present studies to distinguish between these alternative possibilities. Since Gm(x) and Gm(20) are closely associated with Gm(a) in Caucasians and if present occur on the same γ-globulins as Gm(a), it might be expected that if a crossover occurred these might have been transposed along with the unit coding for Gm(a). Studies of Gm(x) in Mongoloid populations indicated that it did not occur in association with the product of the Gm^{fya} gene. Gm(20) on the other hand, although more difficult to test for, did appear in such an association in some but not in all instances. Thus the results with these additional genetic markers have not resolved the question of the type of genetic event leading to the contrasting Mongoloid and Caucasian genes.

The results utilizing enzymatic splitting for the separation of the genetic antigens, along with the relationships in different racial groups suggests that the sequence of antigens from the N terminal portion of the heavy chain might be Gm(f), (y), (a). The Gm(f) and (y) antigens thus far have always been found together on the same polypeptide chain and are always concordant in population studies. They might well have been considered identical if the enzymatic splitting with papain had not occurred between their positions. Special attention was directed to the immunochemical features of Gm(z) as compared to Gm(f). These studies indicated that Gm(z) is probably in a position very similar to Gm(f) on a homologous type heavy chain since it also occurred on the Fd fragment and showed the same unusual characteristics of Gm(f) with regard to requirement of light chain combination for complete expression.

The present study has centered on the genetic antigens of the major γG1-subgroup. Even more antigens have been detected for the minor γG3-subgroup and a single antigen has been identified for the γG2-class (25). It has become apparent that the genetic loci involved in these three types of γ-globulin are very closely linked, perhaps occupying sequential positions on the chromosome. In a single racial group these gene complexes segregate, with only rare exceptions, as one genetic unit. However striking differences in these relationships

are observed in different racial groups. Here again the value of having double markers on the γG1–heavy chains becomes apparent in attempting to understand the variations in the relationship of the genes to each other. Table VI shows the major gene complexes found in three racial groups. The most striking difference noted was the association of the $Gm^{b^0b^1b^4b^5b^3}$ gene with the Gm^{fy} gene in Caucasians and with Gm^{za} in Negroids. This complete shift of positions of genetic markers in relation to one another would certainly appear to be the result of an intergenic crossover between subgroup genes. With a single genetic antigen on the γG1–peptide chain the differences might be explained by a point mutation. The presence of the exchange involving double markers indicates that the Negro-Caucasoid difference is almost certainly due to a crossover.

TABLE VII

Vertical Rows of Gene Complexes Illustrating Four Rare Combinations Contrasted with the Usual Two Types found in Caucasians

	Usual caucasoid		Rare caucasoid			
γG1	a	y	a	y	y	—
	z	f	z	f	f	—
γG3	g	b^0	g	g	g	b^0
		b^1				b^1
		b^4				b^4
		b^5				b^5
		b^3				b^3
γG2		n	n	n		n

Recently two Caucasian families have been studied where a unique gene complex can be followed. Here the Gm^{yf} gene and the Gm^g gene are inherited together in sharp distinction to the usual situation. These gene complexes are shown in Table VII. In one of these families[5] the unusual gene complex is $Gm^{yf} - Gm^g - Gm^n$ in sharp contrast to the usual $Gm^{yf} - Gm^b - Gm^n$. All of the multiple (b) antigens are missing in this unusual situation and are replaced by the (g) antigen. The only logical explanation of such a gene complex is that it arose through a crossover event between the closely linked loci of the γG–subgroups. Table VII also shows another rare gene complex where no markers are detectable for the γG1–subclass in two Caucasian families.[6] The

[5] This family has been studied with Dr. Jacob B. Natvig, Dr. T. Gedde-Dahl, and Dr. T. E. Cleghorn. The second family has been studied with Dr. C. Ropartz and is being published separately.

[6] Details of investigations on these families will be reported separately with Dr. L. Martensson.

explanation of this finding remains obscure but again illustrates the independence of genetic effects within individual subgroup genes.

The availability of different genetic markers for the different subgroups along with studies of various recombinations including those described in the preceeding paragraph, offers the possibility of mapping the genes concerned in the three heavy chain subgroups. The evidence currently at hand from both family and population studies favors the order –γG2–γG3–γG1–. However, the availability of only one genetic marker for the γG2 type hampers complete resolution of this question. Despite the evidence for rare crossovers between the cistrons coding for the proteins of the three γG–subgroups, the more striking finding has been their very close linkage. They may well occupy sequential positions on the chromosome which goes along with the marked antigenic and structural similarity between the proteins involved and may have arisen through relatively recent duplication. It appears very unlikely that a significant number of additional cistrons occupy intermediate positions particularly since the vast majority of the γG–globulin molecules can be accounted for and carry the genetic markers described. It will be of considerable interest to extend these observations to include the genes for the γM– and γA–heavy chains and eventually to obtain a complete map of the cluster of closely linked and partially similar heavy chain genes involved in the γ–globulin system.

SUMMARY

The genetic control of γG1–heavy chains was investigated by taking advantage of two recently described genetic antigens, Gm(z) and Gm(y), both produced by heteroimmunization of rabbits with myeloma proteins. These were studied in conjunction with known genetic markers, Gm(a) and Gm(f). The results indicated that among Caucasians there are two major allelic genes, Gm^{za} and Gm^{fy}, coding for distinct varieties of γG1–heavy chains. Each of these contains a pair of genetic antigens which are located on different fragments of the chain and can be separated by enzymatic splitting with papain. The different areas of the heavy chains appear to be under the control of the same gene. In Mongoloid populations a grouping of three genetic antigens, Gm(f), (y), and (a), was found on isolated myeloma proteins and normal γ–globulins indicating the presence of a Gm^{fya} gene. The possible genetic events leading to the contrasting Caucasian and Mongoloid genes are discussed. In the γ–globulin system the occurrence of multiple genetic antigens in different positions of the same heavy chains is the general rule.

A better understanding of the relationships between the genes for the γG1–subgroup to those for the γG2– and γG3–subgroup has been obtained through the use of the multiple genetic markers. Strong evidence was obtained for intergenic crossover mechanisms to explain racial differences in the relation-

ships of these genes as well as certain unusual gene complexes found through family studies. Further evidence was obtained for mapping the closely linked genes for the three subgroups in a specific order.

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