FURTHER STUDIES ON THE γG-HEAVY CHAIN GENE COMPLEXES, WITH PARTICULAR REFERENCE TO THE GENETIC MARKERS Gm(g) AND Gm(n)

By J. B. NATVIG, M.D., H. G. KUNKEL, M.D., W. J. YOUNT, M.D. and J. C. NIELSEN, M.D.

(From The Rockefeller University, New York 10021, and the University Institute of Forensic Medicine, Copenhagen, Denmark)

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The Gm antigens represent genetic markers for the heavy chains of γG-globulin and are distributed within three out of the four heavy chain subgroups (see references 1–4). These subgroups of heavy chains show distinct antigenic, physiochemical and structural differences indicating that they are controlled by genes at different cistrons (4–7). Previous studies have dealt primarily with the heavy chains of the γG1-subgroup, and the corresponding main γG1-genes, Gm^{za} and Gm^{fy}, and in Mongoloids, Gm^{fya} (8, 9). Furthermore, the various Gm(b) antigens of the γG3-subgroup have been studied in detail (10–12). Recently two new antigens were characterized, Gm(g) (13, 14) of the γG3- and Gm(n) (15) of the γG2-subgroup, which have proven of particular utility for understanding the relationship between the different cistrons involved in γG–heavy chain synthesis.

In the present study details are presented of the characterization and distribution of Gm(g) and Gm(n) in isolated γ-globulins and in whole sera obtained from various populations and families. With the availability of the new Gm antigens more than 95% of the γG-molecules and five separate genes coding for γG–heavy polypeptide chain synthesis can be traced and characterized by genetic markers.

The relationship between these five genes at three different cistrons has proven of particular interest. In addition to certain widely prevalent gene complexes which vary in different populations, a large number of rarer gene complexes have been encountered which appear to have arisen through unusual crossing-over events. Of special interest was the finding of gene complexes that lack the recognizable alleles at one cistron while retaining common types at the other two cistrons.

Materials and Methods

Human Sera.—Normal human sera from various population groups (Caucasian, African Negro, Japanese, Chinese, Easter Island, Asian Indian, and Australoids) were kindly provided by Dr. F. H. Allen, Dr. A. G. Bearn, Dr. K. Berg, and Dr. K. Takatsuki. Some of the family

sera were kindly provided by Dr. R. Ceppellini, Dr. K. Berg, Dr. A. G. Bearn, Dr. T. Gedde-Dahl, Dr. E. van Loghem, Dr. C. Ropartz, and Dr. M. Waller.

Multiple myeloma proteins and macroglobulins were obtained from sera of patients with multiple myelomas or Waldenström's macroglobulinemia. Some myeloma sera were kindly provided by Dr. A. Pick, Dr. H. Fudenberg, Dr. E. van Loghem, Dr. E. Osserman, and Dr. K. Takatsuki. In a few cases, sera used were from patients without clinical disease, but with a sharp "monoclonal" γ-globulin peak on electrophoresis.

Isolation of γ-Globulins, Their Fragments and Chains.—The myeloma proteins or macroglobulins were isolated by zone electrophoresis (16), followed by classification in double diffusion agar with specific antisera for γ-globulin class and γG–heavy chain subgroup. Papain digestion of isolated γ-globulin was carried out according to Porter (17) in 0.01 M cysteine and 0.002 M EDTA and using an enzyme to protein ratio 1:100 (w/w). Digestion was regularly continued for 18 hr at 37°C. However, some γ-globulins were digested very easily and complete splitting was obtained after ½–1 hr. Pepsin digestion was performed at an enzyme to protein ratio 2:100 in 0.1 M acetate buffer at pH 4.1. The digest products were tested as previously described (18).

Reduction of isolated γ-globulins to separate heavy and light chains was carried out for 1 hr with 0.2 M β-mercaptoethanol. This was followed by alkylation with a 10% excess of iodoacetamid and dialysis against 1 M propionic acid. The separation was finally made on a Sephadex G-100 column equilibrated with 1 M propionic acid. Before use, the chains were dialyzed against distilled water and mixed with an equal amount of 0.3 M NaCl.

Genetic Typing.—Gm typings were mostly performed by the hemagglutination inhibition methods with the tube and slide techniques employing anti–Rh-coated red cells (4, 19). The Gm systems included in the tests are shown in Table I. Some anti-Gm reagents of human origin were from rheumatoid arthritis sera (Ragg reagents), others originated from normal sera (Snagg anti-Gm sera) (see Table I). However, the typings were mostly performed with rabbit and primate antisera raised against isolated myeloma proteins carrying a particular genetic antigen. To obtain an anti-Gm reagent, the antiserum was absorbed with excess amounts of a human serum or isolated myeloma proteins negative for the particular genetic antigen. The precipitate was spun down and the supernate used as anti-Gm reagent (20). 100 Caucasian sera independently typed with anti-Gm sera of human, rabbit, and non-human primate origin gave the same results. The specificities of these Gm typing systems thus generally were concordant for anti-Gm antisera of heterologous and isologous origin. Furthermore, the heteroantisera were very sensitive, and behaved as Snagg anti-Gm sera. They were not inhibited by negative normal serum undiluted or only diluted 1:2. Positive sera inhibited in dilutions from 1/40–1/80 for Gm(g), to 1/640–1/1280 for Gm(a) and Gm(f) tests. For each Gm typing system at least one anti-Gm serum was of a Snagg type, thus providing optimal specificity and sensitivity in the tests. The regular typings were done at a serum dilution of 1/10. In all the crucial sera with Gm antigens indicating rare gene complexes the typings were repeated at twofold dilutions starting with undiluted serum.

In addition to this hemagglutination technique with γG–anti–Rh antibodies, some Gm(n), Gm(g), and Gm(b^o) typings were performed by the precipitation technique (15) employing strong heteroantisera with anti-Gm specificity.

No anti-Rh antibody belonging to the γG2-subgroup was found despite the testing of more than 200 high titered incomplete (γG) anti-Rh sera. Gm(n) typing was therefore done by precipitation technique as originally described, or using a new hemagglutination inhibition method with Gm(n+) myeloma proteins coupled by bisdiazotized benzidine (BDB) to red blood cells (21). By this method the γ-globulin was attached to the red cells by stable covalent bonds. The optimal proportion of red cells, of BDB, and of γ-globulin was determined by two dimensional titrations. 1 volume of packed O, human red cells was added to 1 volume of a stock BDB solution diluted 1/15 in phosphate-buffered saline (0.15 M) at pH 7.3. The

TABLE I

Main Test Reagents Used For Gm Typing

Subgroup	Genetic antigen	Anti-Gm	Dilution	Antigen for red cell coating
γG1	Gm(a)	30-70 (H)*	1/16	
		Sm (H)	1/16	
		6 (B)	1/400	Gm(zax) anti-D 30-91 ½ or
		Dc (M)	1/100	Gm(za) myeloma protein
	Gm(z)	9-53 (R)	1/3	
	Gm(x)	M.S. (H)	1/16	Gm(zax) anti-D 30-91 ½
		We (H)	1/4	
	Gm(f)	8-76 (R)	1/200	Gm(f) anti-D 30-83 ½ or
		A.J. (H)	1/16	Gm(f) myeloma protein
γG2	Gm(n)	1 (M)	1/64	
		2 (M)	1/32	Gm(n) myeloma protein
		7-56 (B)	1/4	
γG3		39-78 (H)	1/32	
	Gm(g)	7-55 (B)	1/128	Gm(g) anti-D 47-52 ½ or
		9-32 (R)	1/32	Gm(g) myeloma protein
		7-94 (R)	1/32	
	Gm(b)	35-10 (H)	1/4	
	$Gm(b^0)$	23-57 (H)	1/4	
	Gm(b)	74 (R)	1/200	
	Gm(b)	9-49 (R)	1/32	Gm(b) anti-D 31-09 ½ or
	$Gm(b^1)$	22-47 (H)	1/2	Gm(b) myeloma protein
	$Gm(b^3)$	22-77 (H)	1/4	
	$Gm(b^4)$	Bu (H)	1/5	
	Gm(s)	26-24 (H)	1/2	Gm(st) anti-D Vai ½
	Gm(t)	29–39 (H)	1/2	
	$Gm(c^3)$	S (H)	1/3	Gm(c) anti-D Wa ½
	$Gm(c^5)$	W (H)	1/5	

Due to scarcity of reagents, Gm(s) and (t) were tested on selected samples; some (s) and (t) typings were also kindly performed by Dr. E. van Loghem.

No reagent for Gm(y) was available, but the Gm(y) was recorded in the figures according to data given in the literature (9).

* H, of human origin; R, of rabbit origin; B, of baboon origin; and M, of cynomologous monkey origin.

The following reagents were kindly provided from other laboratories: Anti-Gm(f) A.J. and anti-D Vai from Dr. L. Mårtensson, anti-Gm (b^0) (b^1) (b^3), anti-Gm(s) and (t) from Dr. E. van Loghem and partly from Dr. C. Ropartz, anti-Gm(b^3) and (b^4) and anti-D Wa from Dr. A. G. Steinberg, and anti-Gm(c^3) and (c^5) from Dr. H. Borel.

mixture was left for 2 min, and 10 volumes of γ-globulin at 1–2 mg/ml was added. After 15 min, the coated cells were washed, and used for genetic typing in the same way as the anti-Rh-coated red cells. Controls were always run to see that the coated cells were not agglutinated by normal human sera.

For Gm(n) testings the same myeloma protein (Ne) was used for red cell coating as had

been used to raise the anti-Gm(n) antiserum in animals. To avoid reaction with individual specific determinants (22) the antiserum was absorbed with pepsin-digested Ne protein at 1 mg/ml. These $F(ab')_2$ fragments of Gm(n) molecules did not remove any anti-Gm(n) activity. A similar approach, was particularly suitable for Gm antigens belonging to subgroups where isolated myeloma proteins are rare, for example for Gm(g). Thus a single myeloma protein could be utilized first for immunization to obtain anti-Gm antibodies, and thereafter as carrier of the genetic antigen for red cell coating.

Quantitative Radial Diffusion Technique.—The concentrations of γG3-globulins in whole serum were determined by radial immunodiffusion (23). Before use, antiserum was absorbed with γG1- and γG2-myeloma proteins of homologous light chain type. It was mixed with molten 2% agarose agar and 0.1 M veronal buffer pH 8.6 in a 56°C constant temperature bath in volume ratios 1:5:4. The agar plates were prepared and 5 μl of the human sera and γG3-globulin standard controls respectively were applied in wells 2.8 mm in diameter. After incubation for 16 hr at 37°C, the plates were photographed and the ring diameters measured on the photographic plates. The γG3-concentrations were determined from the standard curve prepared by plotting precipitin ring diameter against log concentration of six γG3-standards (23).

RESULTS

The Gm(g) Antigen.—Besides the original anti-Gm(g) antibody from a rheumatoid arthritis serum, sera from one baboon (7-55) and four different rabbits (7-94, 7-95, 9-31, and 9-32) immunized with the Gm(g) myeloma protein Vi showed anti-Gm(g) specificity after proper absorption with Gm(g−) human sera or myeloma proteins. These represented all the possible immune sera against the Gm(g) protein and they were all positive. Gm(g) thus appeared to be a strong antigen in rabbits and primates. It was apparent that as soon as the antisera were useful for γG3-subgrouping in precipitation, they also worked for Gm(g) typing in hemagglutination tests. The working titers used, however, varied considerably, from 1/8 to 1/256, and the strongest antisera also detected the Gm(g) antigen with precipitation techniques.

Several hundred sera of Caucasian, Negro, and Mongoloid population groups representing all the known variations in Gm phenotype, different myeloma proteins, their fragments and chains were typed with concordant results when comparing the original human anti-Gm(g) and the antisera of heterologous origin. The specificity of the reactions were not changed when the anti-Gm(g) antisera were absorbed with excess amounts of Fab fragments of Gm(g) myeloma proteins, removing all anti-Fab activity. The distribution of Gm(g) and other Gm antigens in a panel of 250 Caucasian sera is shown in Table II. Although most sera were either Gm(z+a+g+) or Gm(z−a−g−), the few exceptions were of special significance. In the present unselected material and other selected Caucasian sera, nine Gm(z+a+g−) and three Gm(z−a−g+) individuals were detected. In these exceptional sera the inhibiting capacities for Gm(g) were the same whether Gm(a) was present or not, and vice versa. Gm(g+) sera showed inhibition in dilutions up to 1/40–1/80 and Gm(a) sera in dilutions 1/640–1/1280. Gm(a) and Gm(g) were also usually inherited together in

TABLE II

Gm Types among 250 Caucasian Sera

Gm phenotype	Genotype	No. of sera
z−a−g−f+b+	$Gm^{f}Gm^{b}/Gm^{f}Gm^{b}$	123
z+a+g+f+b+	$Gm^{za}Gm^{g}/Gm^{f}Gm^{b}$	101
z+a+g+f−b−	$Gm^{za}Gm^{g}/Gm^{za}Gm^{g}$	22
z+a+g+f+b−	$Gm^{za}Gm^{g}/Gm^{f}Gm^{g}$	1
z+a+g+f−b+	$Gm^{za}Gm^{g}/Gm^{za}Gm^{b}$	2
z+a+g−f+b+	$Gm^{za}Gm^{b}/Gm^{f}Gm^{b}$	1

Frequencies of gene complex: $Gm^{za}Gm^{g}$:0.30
$Gm^{f}\ Gm^{b}$:0.69
$Gm^{f}\ Gm^{g}$:0.002
$Gm^{za}Gm^{b}$:0.006

TABLE III

Number of Myeloma Proteins of Different Gm Types with Particular Reference to Gm(g)

Class and Gm type	No.	Gm(g)	Lowest protein concentration mg/ml inhibiting the Gm(g) test
γG1 a+z+	10	—	>1
f+	10	—	>1
a+f+*	1	—	>1
γG2 n+	5	—	>1
n−	5	—	>1
γG3 b^0+, b^1+, b^3+, b^4+	16	—	>1
b^0+, b^1−, b^3+, b^4−‡	1	—	>1
b^0+, b^1+, b^3−, b^4+§	1	—	>1
g	7	+	0.008
γG4−	5	—	>1
γA1−	10	—	>1
γA2−	2	—	>1
γM−	10	—	>1

* Chinese.
‡ Japanese.
§ Negro.

families studied, but the combinations of Gm(z+a+g−) and of Gm(z−a−g+) in certain families were stable and inherited. In the latter case the Gm(g) antigen was associated with Gm(f) and Gm^{f} and Gm^{g} inherited as a gene complex. Thus the Gm(g) antigen was usually inherited together with Gm(za) in

Caucasians, but Gm(g) behaved as a separate antigen which could segregate from Gm(a). This was also evident from the Gm typing of isolated myeloma proteins and by further population studies.

Molecular Localization of Gm(g).—Isolated myeloma proteins and macroglobulins were tested for the presence of the Gm(g) antigen employing one rabbit (7-94), one baboon (7-55), and one human (39-78) anti-Gm(g). The results are shown in Table III. Out of 22 γG3-myeloma proteins of Caucasian origin, six were Gm(a−z−f−b−g+n−), and 16 were Gm(a−z−f−b+g−n−). Two proteins were of Mongoloid origin; one was Gm(g+) and negative

TABLE IV

Lowest Protein Concentration (mg/ml) of Whole Myeloma Proteins and Respective Isolated Fragments and Chains Giving Inhibition

Protein tested for inhibition	Gm(g) test		Gm(n) test
	Vi*	Pr‡	Ne§
Whole M. Protein	0.008	>1	0.015
Papain Fc	0.004	>0.5	0.004
Papain Fab	>0.5	>0.5	>0.5
Pepsin $F(ab')_2$	>0.5	>0.5	>0.5
Heavy Chain	0.004	>0.5	0.004
Light Chain	>0.5	>0.5	>0.5
Octadecapeptide‖ (C-terminal)	>0.5		

* Isolated γG3-myeloma protein Vi-Gm(a-z-f-b-g+n-).

‡ Isolated γG3-myeloma protein Pr-Gm(a-z-f-b+g-n-).

§ Isolated γG2-myeloma protein Ne-Gm(a-z-f-b-g-n+).

‖ Tested in a concentration corresponding to 0.5 mg/ml of the whole molecule.

for all the other Gm antigens, the other was Gm(b^0+, b^1−, b^3+, b^4−). One γG3–Negro myeloma protein was Gm(b^0+, b^1+, b^3−, b^4+). All the isolated myeloma proteins of γG1-, γG2-, γG4- and γA-class and the γM-globulins were Gm(g−).

In a panel of 100 high titered incomplete anti-Rh antisera two of them were found to coat selectively for Gm(g) typing with anti-Gm(g), while no reaction was obtained with anti-Gm sera of other specificities including Gm(b). In both instances the coating γ-globulin was shown to be of the γG3-subgroup by specific rabbit antisera to the γG3-subgroup, and by specific human antisera to the $F(ab')_2$ fragments. The latter antisera directed against subgroup determinants in the Fab fragment can be used for subgrouping of specific antibodies (24). Anti-Rh antibodies from three donors, one of them the anti-CD Ripley, contained Gm(g) among other Gm antigens.

Gm(g) was exclusively restricted to the γG3-subgroup in myeloma proteins and in isolated anti-Rh antibodies. All γG3-myeloma proteins were either

Gm(g+) or Gm(b+), and no isolated protein was simultaneously positive for Gm(g) and any of the Gm(b) antigens, b^0 b^1 b^3 b^4 c^3 c^5.

The Gm(g) myeloma protein, Vi, was utilized for detailed molecular characterization of the Gm(g) antigen. The Gm(g) activity was found in the Fc fragment but not in the Fab fragments obtained after enzymatic digestion with papain (Table IV). The Gm(g) protein was very easily split by papain, and after 18 hr of splitting no Fc fragment was recovered. The papain digestion in the present experiments were run only 1/2 hr, which gave a complete splitting and a definite Fc fragment, although the yield was relatively low. The full inhibiting capacity on a molar basis was also recovered from the isolated heavy chain, while no inhibition was obtained by the isolated light chains. Similar fragments and chains of a γG3-Gm(b) myeloma protein (Pr) were studied as a control, and did not inhibit the Gm(g) system.

Heavy chain subgroup specific antigens are known to occur in the Fd part of the γG3-chains as well as in the Fc portion. Attempts were thus made to detect a Gm-genetic antigen in the Fd fragment. Extensive immunization of rabbits with Fab and Fd fragments of Gm(g+) and Gm(b+) myeloma proteins resulted in some antisera with γG3-specificity. However, no anti-Gm activity was revealed. Also absorption of the very strong anti-Gm(g) antisera 7-55 and 9-32 with Fc fragments of the Gm(g) protein Vi abolished all activity for genetic antigens. The Gm(g) antigen was thus exclusively recovered from the Fc part of the heavy chains.

Testing of Peptide Fragments of γG-Globulin Heavy Chains.—Some C-terminal octadecapeptides obtained by cyanogen bromide splitting were sent by Dr. Prahl for Gm studies (6). The octadecapeptide of the Gm(g) chain (Vi) tested in a molar concentration corresponding to 0.5 mg/ml of the intact protein did not show any inhibition of the anti-Gm(g) reagents of animal (9-32 and 7-55) or human origin. The anti-Gm antisera utilized were absorbed with excess amounts (1–3 mg/ml) of homologous $F(ab')_2$ fragments. An attempt to couple red cells with the octadecapeptide by bisdiazotized benzidine for subsequent agglutination by specific antisera also failed to demonstrate the Gm(g) antigen. The octadecapeptide of the Gm(b) chain (Zu) did not inhibit several anti-Gm(b) reagents including the specificities Gm(b^0), (b^1), (b^3), (b^4). Also C-terminal octadecapeptide from a Gm(a) heavy chain (Da) did not inhibit various human anti-Gm(a) reagents. The antigens tested for were all present in the respective whole heavy chains and their Fc fragments, since they completely inhibited the respective anti-Gm antisera.

The Relationship between Gm(g) and Various Gm(b) Markers.—A series of Gm(b) markers (b^0, b^1, b^3, b^4, b^5, s, t, c^3, c^5) along with Gm(g) make up the Gm antigens of the γG3-subgroup. The relationship between the new γG3-antigen Gm(g) and a series of Gm(b) markers were studied in sera from different populations. Gm(g) was first compared with Gm(b^0), as shown in Table V. All the

sera tested from the Caucasian, the Negro, the various Mongoloid population groups and Australoids (Aborigines) were either Gm(g+) or Gm(b^0+) or positive for both Gm(g) and Gm(b^0). Gm(g) was thus always present when Gm(b^0) was lacking and vice versa. The variation in Gm(g) frequency differed strikingly and ranged from zero in the pure Negro population to 100 % in the

TABLE V

Gm Antigens g and b^0 of the γG3-Subgroup

Gm(g)	Gm(b^0)	Caucasian (406)	Negro (123)	Chinese (124)	Easter Island (156)	Japanese (188)	Asian Indians (68)	Australoids (55)
+	+	153	0	37	72	92	32	0
−	+	219	123	78	60	23	5	0
+	−	34	0	9	24	73	31	55
Gene frequencies of Gm^g.....		0.27	0	0.22	0.35	0.63	0.69	1.00

TABLE VI

Selected Gm(b) Subtypes Among 70 Nigerian Negro and 188 Japanese Sera Typed with Different Gm(b) Antisera

g	b^0	b^*	b^1	b‡	b^3	b^4	c^3	c^5	Negroes	Japanese
−	+	+	−	−	+	−	−	−	0	15
−	+	+	+	+	+	+	−	−	31	0
−	+	+	+	+	−	−	+	+	5	0
−	+	+	+	+	−	+	+	+	1	0
−	+	+	+	+	+	+	+	−	9	0

Some of the sera were kindly typed for Gm(s) and (t) by Dr. Erna van Loghem, and simultaneously lacked these factors and Gm(g).

* Anti-Gm(b) of heterologous origin obtained from three different rabbits. Two were immunized with a Caucasian Gm(b) myeloma protein and one with baboon γ-globulin.

‡ Original Gm(b) system J.K. (40) and two other R.A. anti-Gm sera, Bo and 31-67. (see text).

Australoids. In all the sera and myeloma proteins studied Gm(b^0) was the only antigen which was common to all Gm(b) molecules and Gm(b) sera. In contrast several Gm(b^0+) sera lacked some other Gm(b) antigens together with Gm(g). 15 out of the 188 Japanese sera studied were Gm(b^1-, b^4–, g-) (Table VI). Among 70 Nigerian Negro sera five were Gm(b^3-, b^4-, g-) and one was Gm(b^3-, g-). In Gm(b^0) homozygous Caucasians also Gm(c^3), (c^5), and (s) were absent together with Gm(g). Since Gm(b^0) was the only genetic antigen which was always present when Gm(g) was absent it appeared that Gm(g) and Gm(b^0) corresponded most closely as antithetic markers of two major types of heavy chain within the γG3-subgroups.

Specificity of Anti-Gm(b) Reagents from Rheumatoid Arthritis Sera and from Heteroimmunization.—The original anti-Gm(b) antibody (40) from a rheumatoid arthritis (R.A.) patient and two other anti-Gm sera of similar origin (31-67 and Bo) typed human sera as anti-Gm(b^1) when compared for various anti-Gm(b) specificities. All of the sera positive for Gm(b^1) were positive with the three R.A. Gm(b) systems, while some Gm(b^1) sera lacked either Gm(b^3, b^4, c^3, c^5, or s) (see Table VI). In contrast, all the sera negative for Gm(b^1) were negative with the three R.A. Gm(b) systems.

Three different anti-Gm(b) reagents of heterologous rabbit origin, all typed as anti-Gm(b^0) (Table VI). All sera tested were either positive for both types of reagents or negative in the same tests. One of these antisera (R 9-49) was raised against γ-globulin from a non-human primate (baboon) showing that Gm(b^0) was present in the primate γ-globulin. Gm(b^0) thus appeared to be a strong antigen in rabbits.

The heterospecific anti-Gm(b) antisera were absorbed with Gm(b^0+) human sera lacking one or more of the antigens b^1, b^3, b^4, or b^5 to try and detect these antigens with heteroantisera. However, after absorption the antisera failed to show any anti-Gm activity. Also, similar absorptions of rabbit and primate anti-Gm(g) antisera with human sera of various phenotypes and population groups failed to show any heterogeneity of the Gm(g) antigen.

The Gm(n) Antigen Detected by Indirect Hemagglutination.—Besides the original precipitation technique (15), extended family studies on Gm(n) have been performed employing a new indirect hemagglutination technique (7). Sera of various phenotypes and population groups were tested by both methods with full concordance, and the Gm(n) antigen behaved as a simple codominant Mendelian trait. In the present study the molecular localization of Gm(n) was investigated with the original anti-Gm(n) antiserum, and two new but weaker antisera against a Gm(n+) heavy chain and whole myeloma protein respectively. They were raised in cynomologous monkeys and a baboon. Five Gm(n+) myeloma proteins all exhibited strong inhibition, while five Gm(n−) γG2-proteins exhibited no inhibition of all the anti-Gm(n) antisera. The Gm(n) activity was retained in the Fc fragments and in isolated heavy chains from Gm(n+) proteins. No inhibition was obtained with Fab fragments or the light chains from the same proteins (see Table IV). Several isolated γG1-, γG3-, γG4-, γA-, and γM-globulins showed no inhibition of the new Gm(n) typing system.

Relationship between the Gm Markers in Population Groups.—As reported earlier Gm antigens belonging to three different subgroups and the corresponding γG-heavy chain genes are closely associated making up certain gene complexes (1, 7). However, the degree of association seems to differ. The two new antigens Gm(g) and Gm(n) were used for more extensive studies of this relationship. The γG1-markers Gm(za) and Gm(f) and the γG3-markers Gm(b^0) and Gm(g) showed a uniquely stable degree of association. With only 18 exceptions,

5000 Caucasian sera all had Gm(za) associated with Gm(g) and Gm(f) associated with Gm(b⁰) (7). In 123 Negro sera Gm(za) was universally associated with Gm(b⁰) (Table VII). In Mongoloid sera Gm(za) was associated either with Gm(g) as in Caucasians or with Gm(b⁰) as in Negroes and Gm(fa) was related to Gm(b⁰). The respective gene complexes appeared to be as stable as the corresponding Causcasian types. This was indicated from family studies in an Easter

TABLE VII

Gm(n) Type in Adult Individuals Homozygous for Various Gm Gene Complexes Involving γG1 and γG3

Subgroup and Gm antigen						Detected gene complex	Caucasian (400)*	Negro (123)	Easter Island (156)	Japanese (188)	Asian Indian (68)	Australoid (54)
γG_1			γG_3		γG_2							
z	a	f	g	b^0	n							
+	+	−	+	−	+	zagn+	3	—	1	4‡	3	4
					−	zagn−	31	—	13	40	25	50
−	−	+	−	+	+	f bn+	191	—	8	—	2	—
					−	f bn−	28	—	0	—	0	—
+	+	−	−	+	+	zabn+	—	0	—	0	0	—
					−	zabn−	—	123	—	15	2	—
−	+	+	−	+	+	fabn+	—	—	46	—§	—	—
					−	fabn−	—	—	2‖	—	—	—

* The figures in brackets give the total number of sera tested.

‡ Some Japanese sera of this type were negative with the original human anti-Gm(f) but weakly positive with a heterospecific anti-Gm(f) possibly indicating a heterogeneity of Gm(f).

§ One heterozygous Gm^{za} Gm^{b}/Gm^{fa} Gm^{b} individual was Gm(n−) indicating a Gm^{fa} Gm^{b} Gm^{n-} gene complex.

‖ Two children also had this phenotype.

Island population with a high incidence of typical Mongoloid gene complexes and where no exchange with crossing-over was detected between γG1- and γG3- genes respectively.

In contrast, the Gm(n) antigen showed a considerable degree of variability, and it appeared that Gm(n) relatively frequently occurred in many different combinations with other Gm markers. The relationship between the Gm(n) marker (γG2) and the Gm markers of the γG1- and γG3- subgroups was most clearly demonstrated in sera that were homozygous for the Gm antigens of the γG1- and γG3- subgroups as shown in Table VII.

The Gm^{za} Gm^{g} Gm^{n+} complex was found in five different populations. The frequencies of Gm^{n+} and Gm^{n-} in Gm^{za} Gm^{g} homozygous individuals were ap-

proximately 0.05 and 0.95 respectively both in the Caucasian and Mongoloid populations. In Caucasian $Gm^{f}\ Gm^{b^0}$ homozygous individuals, the frequencies of Gm^{n+} and Gm^{n-} were approximately 0.75 and 0.25 respectively. Here, data from Easter Island and Asian Indian populations were too scanty to be significant. The Negro $Gm^{za}\ Gm^{b}$ gene complexes all lacked Gm^{n+}. This was also true for the Mongoloid $Gm^{za}\ Gm^{b}$ complex although only a relatively small number of these gene complexes had been tested. However, 50 heterozygous $Gm^{za}\ Gm^{b}/Gm^{za}\ Gm^{g}$ Mongoloid individuals were all Gm(n−) giving further support that $Gm^{za}\ Gm^{b}$ very rarely if ever is linked with Gm^{n+}.

The Mongoloid $Gm^{fa}\ Gm^{b}$ gene complex was most frequently associated with Gm^{n}. However, four Easter Island individuals homozygous for $Gm^{fa}\ Gm^{b}$ were Gm^{n-}. The Gm(x) antigen was used as an additional marker for γG1, usually associated with Gm^{a}. All the $Gm^{fa}\ Gm^{b}$ homozygous individuals lacked the Gm (x) marker. Also all the $Gm^{za}\ Gm^{b}$ homozygous Negroes and Mongoloids in this study were Gm(x−). Among the $Gm^{za}\ Gm^{g}$ homozygous individuals, Gm (x+) was found with the same frequency whether Gm (n) was present or not. One Caucasian, $Gm^{f}\ Gm^{b}$ homozygous, was Gm (x+) and Gm(n+) indicating a $Gm^{xf}\ Gm^{b}$ and possibly also Gm^{n+} gene complex.

Gene Complexes which Lack both Common Alleles in One Cistron.—Several Caucasian families with rare gene complexes were studied in detail with regard to the relationship among γG1-, γG2-, and γG3- genetic antigens. This resulted in demonstration of recombination between γG–heavy chain cistrons which has been reported separately (7, 25). In 16 out of the 18 families kindly provided from various laboratories for additional studies, Gm(g) and Gm(n) showed regular inheritance of rare gene complexes and the recombination frequency between the γG-subgroup cistrons was estimated to be extremely low. However, in some of these 16 families the unusual gene complexes lacked both common alleles Gm^{b^0} and Gm^{g} of the γG3-cistron while γG1- and γG2-markers were present. For this study the Gm(g) and Gm(n) antigens were crucial.

The first family B9-68 (Fig. 1), showed two children with an unusual phenotype Gm (z+ a+ g+ f+ b− n+) indicating a gene complex $Gm^{f}\ Gm^{n+}$ which lacked Gm^{b}. All the children had a Gm^{za} (or Gm^{zax}) $Gm^{g}\ Gm^{n-}$ gene complex from the father. Thus from the children's phenotype it was not possible to determine whether Gm^{g} was also connected to the $Gm^{f}\ Gm^{n+}$ complex. However, the mother was Gm (a− g− f+ b+ n+) which gave further information. Since she was Gm (g−) her $Gm^{f}\ Gm^{n+}$ gene complex also lacked Gm^{g}. The sera of the children were repeatedly tested for inhibition of anti-Gm (b) and the mother's serum for anti-Gm (g) inhibition. However, they showed no inhibition even with undiluted serum. The sera were tested for all the different subfactors of Gm (b) available, and both children lacked b^0, b^1, b^3, b^4, s, t, c^3, and c^5. It thus appeared that this family contained a gene complex $Gm^{f},—,Gm^{n+}$ lacking both common alleles Gm^{b} and Gm^{g} of the γG3-cistron.

The second family showed two siblings with phenotypes indicating the rare gene complex Gm^{f},—,Gm^{n+} (Fig. 2). In the branch to the right (J) the father

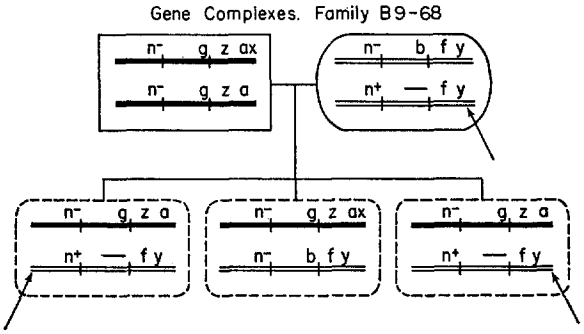

FIG. 1. A Danish family with the unusual gene complex Gm^{n+}, —, Gm^{f} in the mother and two children. The mother's phenotype showed that the complex lacked Gm^{g}, and the phenotype of the two children that Gm^{b} was absent (see text). Gm (a, b, and f) typing of the family has been published by Henningsen (36).

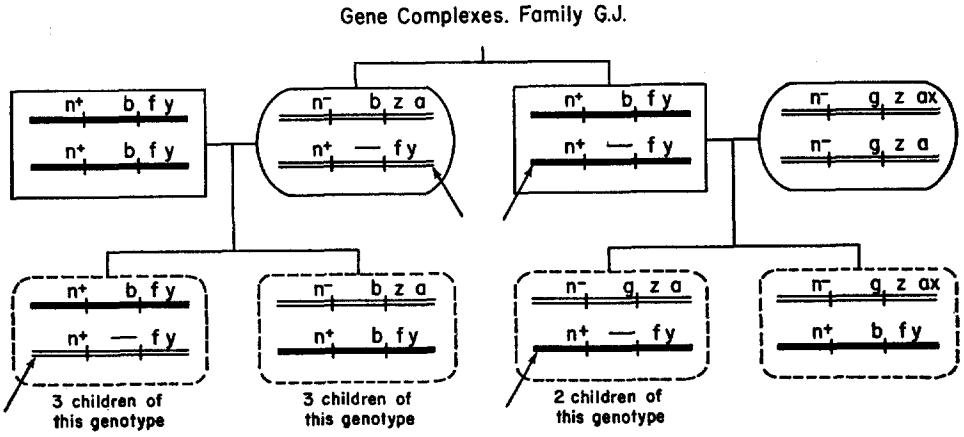

FIG. 2. A Danish family with a sister and brother with the rare Gm^{n+}, —, Gm^{f} gene complex. Two of the brother's children and three of the sister's children appeared to have the rare gene complex. Also, in the sister and three of her children a rare $Gm^{n-}Gm^{b^0, st, b^3}$, Gm^{za} gene complex was present (see text). Gm (a, b, and f) typing of this family has been published (37, 38).

had a gene complex Gm^{f},—,Gm^{n+} without Gm^{g} and it was indicated from two children that this gene complex also lacked all Gm (b) antigens. The father's serum was Gm (g—) and the two children's sera Gm (b—) even when testing the undiluted sera. Another child had inherited a regular Gm^{f} Gm^{b} Gm^{n+} gene

complex from the father. The mother of the left branch of the family (G) had, with high probability, the same Gm^f,—,Gm^{n+} complex. This mother also presented a rare Gm^{za} Gm^b Gm^{n-} complex which was inherited by three of her children. From the mother's phenotype it was not possible immediately to see

TABLE VIII

Record of Caucasian Families with Rare Gm Gene Complexes

Family designation	Reference*	Gene complexes indicated by additional Gm^g and Gm^n Study
		γG1, γG3, γG2
B9-68	(36)	Gm^{fy}, —, Gm^{n+}
G.J.	(37, 38)	Gm^{fy}, —, Gm^{n+} and Gm^{za} Gm^b Gm^{n-}
La	Ropartz	Gm^{fy}, —, Gm^{n+}
71	Ceppellini	Gm^{fy}, —, Gm^{n+}
So	van Loghem	Gm^{fy}, —, Gm^{n+}
B2-68	Ropartz (39)	$Gm^{fy}Gm^g$ Gm^{n-}
Wen	van Loghem	$Gm^{fy}Gm^g$ Gm^{n-}
1	(7, 25)	$Gm^{fy}Gm^g$ Gm^{n-}
H.W.	(7, 25)	$Gm^{fy}Gm^g$ Gm^{n+}
Ha	Waller	$Gm^{fy}Gm^g$ Gm^{n+} or Gm^{fy}, —, Gm^{n+}
De R	van Loghem	$Gm^{fy}Gm^g$ Gm^{n+} or Gm^{fy}, —, Gm^{n+}
85		$Gm^{za}Gm^b$ Gm^{n-}
E 24	Gedde-Dahl	$Gm^{za}Gm^b$ Gm^{n-}
Zu	van Loghem	$Gm^{za}Gm^b$ Gm^{n-}
Vie	van Loghem	$Gm^{za}Gm^b$ Gm^{n-}
EK	Mårtensson and van Loghem	—, Gm^b Gm^{n+}
KN	Mårtensson and van Loghem	—, Gm^b Gm^{n+}
Kr	Mårtensson and van Loghem	—, Gm^b Gm^{n-}

—, indicates gene deletion at one subgroup cistron.

* The families from Dr. C. Ropartz and Dr. M. Waller were previously studied for Gm(a, b, and f), some of the families from Dr. L. Mårtensson and Dr. E. van Loghem also for Gm(g). In the families So, Ha, De R, Zu, and Vie only a few crucial sera were tested in the present study.

that Gm (b) was absent from her Gm^f,—,Gm^{n+} complex, nor could this be derived from the phenotypes of her children. However, the mother had a Gm^{b^0, st, b^3} gene without the Gm (b^1) and (b^4) markers. This gene is seen in Mongoloids, but very rarely in Caucasians (4). If Gm^{b^0} was present with Gm^f Gm^{n+} it would, with very high probability, have included the Gm (b^1) and (b^4) markers which are almost universally present with Gm^f Gm^b in Caucasians. In contrast to the question of the lack of Gm^b in the brother and his children, there

was ample evidence for the same Gm^{f},—,Gm^{n+} gene complex being present in both branches of the family.

By including families kindly supplied by Doctors R. Ceppellini, T. Gedde-Dahl, E. van Loghem, L. Mårtensson, C. Ropartz, and M. Waller, three more families were detected with the same Gm^{f},—,Gm^{n+} gene complex as recorded in Table VIII. Five families thus had a Gm^{f},—,Gm^{n+} gene complex. Three families appeared to have $Gm^{f}\ Gm^{g}\ Gm^{n-}$, one family $Gm^{f}\ Gm^{g}\ Gm^{n+}$ and two other ones $Gm^{f}\ Gm^{g}\ Gm^{n+}$ or Gm^{f},—, Gm^{n+}. Four families showed a $Gm^{za}\ Gm^{b}\ Gm^{n-}$ gene complex. The gene complexes of one of the latter families as indicated by the serum phenotypes is shown in Fig. 3. The mother appeared to have a rare gene complex Gm^{za} lacking Gm^{g} since she was Gm (g—). From the second child it

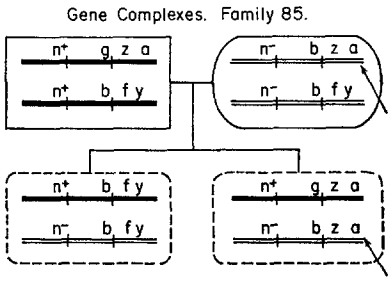

FIG. 3. A Danish family with a mother and one child carrying the rare gene complex $Gm^{n-}Gm^{b}Gm^{za}$. The phenotype of the mother indicated that Gm^{g} was absent, and the Gm types of the children that Gm^{b} was present with Gm^{za} (see text).

is demonstrated that Gm^{za} is associated with Gm^{b}. In contrast to family G. J., the Gm^{b} in this family is a regular Gm^{b^0, b^1, b^3, b^4} gene. In addition, two families showed a gene complex —, $Gm^{b}\ Gm^{n+}$ and one a complex—, $Gm^{b}\ Gm^{n-}$ (9).[1] Thus several families were detected with gene complexes that appeared to lack both the common allelic genes of the γG3-subgroup cistron while products of the two other cistrons were present, while other families showed isolated lack of γG1-genes. This could be due to a third silent allele or to a deletion of a gene. Further studies were performed to try and elucidate this question.

Quantitation of γG3-Protein.—Experiments were performed to characterize the rare gene complexes with Gm^{f},—,Gm^{n+}. Sera from the individuals with the genotype $Gm^{za}\ Gm^{g}\ Gm^{n-}/Gm^{f}$,—,$Gm^{n+}$ were titrated for their Gm (f)- and Gm (n)-inhibiting capacities. In these sera the only Gm (f) and Gm (n) activity originated from the proteins induced by the rare gene complex. Their inhibiting capacity was very similar to that obtained in regular Gm (f+ b+ n+) sera, indicating that the production of γG1-and γG2-heavy chains was normal.

[1] Mårtensson, L., E. van Loghem, and H. G. Kunkel. Unpublished observations.

Furthermore, the sera were tested for inhibition of anti-Gm (g) and anti-Gm (b) respectively. The inhibition was in the lower range of the normal. Also inhibitions of γG3-subgroup specific antisera were low with the sera tested. However, this inhibition test is rather insensitive for quantitative determination. To obtain more thorough quantitation radial diffusion in agar was performed. The results from the sera available for the quantitation are presented in Table IX. They clearly indicate that the concentration of γG3-globulin was distinctly lower in the sera of individuals lacking the two common allelic γG3-genes in one of their gene complexes than in the controls. The normal Caucasian homozygous Gm(b, b) individuals had γG3-levels nearly twice as high as family members with

TABLE IX

γG3-Levels in Normal Sera vs. Sera with a Possible Deletion at the γG3-Cistron

Sera tested (Gm types in parentheses)			Normal	Possible deletion	*P* value
Normal Caucasian (b; b)	vs.	family members (b; —)	1.19 ± 0.54* (62)	0.56 ± 0.14 (5)	<0.001
Normal Caucasian (g; g)	vs.	family members (g; —)	0.62 ± 0.23 (45)	0.36 ± 0.08 (7)	<0.02
Normal family members	vs.	family members with deletion	0.85 ± 0.26 (19)	0.47 ± 0.15 (12)	<0.001

* Mean levels in mg/ml ± 1 SD.

Gm(b, −). Similarly, outstanding differences were observed when comparing normal Gm(g, g) with Gm(g, −) individuals, and all normal family members with all those having Gm (b, −) or Gm(g, −). The differences were statistically significant with *P* values <0.02 and <0.001 respectively. The higher γG3-concentration among Gm(b, −) than of Gm(g, −) members were in accordance with previous findings that Gm(b) chains probably are synthesized at a higher rate than Gm(g) chains (23).

DISCUSSION

The present studies add further evidence for the concept that the heavy chains of the γG3-subgroup are of two major types, one carrying Gm(g) the other Gm(b^0). These chains are coded for by a gene Gm^g, or by a gene Gm^{b^0} together with a variety of other (b) markers. The Gm^g and Gm^{b^0} genes behave as alleles and both markers reside in the Fc part of γG3-chains. However, it is not known whether the Gm(g) and (b^0) markers are localized to homologous positions within the gene. No genetic antigen of the Fd part of either Gm(g) or

Gm(b^0) γG3-heavy chains has been detected although extensive immunization of rabbits with γG3 Fd and F(ab')$_2$ fragments was carried out.

Several studies indicate that γ-globulin genetic antigens reflect amino acid interchanges in the primary structure of the chains. In the case of the Inv antigens of the light chains the interchange of one amino acid, valine, for leucine determines Inv (b) and Inv (a) respectively (26). Gm (a) is, however, determined by two amino acids, aspartic acid and leucine instead of methionine and glutamic acid in Gm (a−) molecules (27, 28). It has been difficult to make heteroantisera that detect the Inv antigens, possibly because they are determined by only one amino acid. The Gm (a) antigen can easily be detected with heteroantisera employing hemagglutination techniques, however not with precipitation methods so far. In contrast, Gm (g), Gm (b), and Gm (n) could all be detected in precipitation tests by heteroantisera, Gm (n) most readily, suggesting that several amino acid interchanges are responsible for these antigens, all of which are restricted to the Fc part. This may add to the production of lattices to provoke precipitation. For the Gm (b) system there should be at least five interchangeable amino acid positions to explain all the b antigens and a number of this magnitude may be involved in Gm (g) and Gm (n).

Some attempts were made to localize the Gm (g) and Gm (b) antigens further. C-terminal octadecapeptides were shown by Prahl (6) to differ in position 11 with phenylalanine in Gm (b) and tyrosine in Gm (g) chains. However, the negative findings with lack of inhibition by the octadecapeptides do not exclude the possibility that amino acid sequences in these peptides may reflect structures responsible for the respective Gm antigens. Such small peptides may well have lost the antigenicity they possessed in the intact Fc fragments.

Attempts to show a mosaicism of the Gm (g) antigen like that of Gm (b) have failed, although absorptions of various anti-Gm (g) antisera were made with sera of various population groups and phenotypes. However, Gm (b) subtypes could not be detected with heteroantisera either. Also, structures in the Gm (g) chain which correspond to some of the Gm (b) subfactors may be shared in common with other subgroups. It has recently been shown[2] that an antigen which parallels the "non-a" peptide (27) is shared by Gm(f), (b), (g), (n+), and (n−) heavy chains, and thus is distributed within three different γG-subgroups. Similarly, (non-b) antigens may be present in other proteins than Gm(g). Furthermore, a (non-g) antigen is found which is shared by Gm(b) proteins and all γG2-globulins but absent in other γ-globulins[2]. This raises the possibility that the Gm(g) and (non-g) structures are localized to homologous positions within the Gm(g) and Gm(b^0) chains and the corresponding genes.

The gene complexes comprised by the γG1-, γG2-, and γG3-cistrons have

[2] Natvig, J. B., H. G. Kunkel, and F. Joslin. Delineation of two antigenic markers, "non a" and "non g," related to the genetic antigens of human γ-globulin. Manuscript in preparation.

undergone both inter- and intracistronic recombinations during evolution (7–9). This was indicated by the variability of gene combinations at the three cistrons. Since many of these changes included several Gm markers they can not be explained by single point mutations. For example, the Gm^{za} Gm^{g} complex in Caucasians and the Gm^{za} $Gm^{b^0, b^1, b^3, b^4, b^5}$ complex in Negroes differ by six Gm markers, each in all probability representing one or more specific amino acids. Similar arguments are valid also for the other combinations of γG1- and γG3-genes. Only one Gm marker, Gm(n), is known for γG2. However, Gm(n) is found in association with all types of γG1- and γG3-markers except in the case of the Negro and Mongoloid Gm^{za} Gm^{b} complex, and Gm(n) probably represents more than one amino acid interchange. Since both Gm^{f}, Gm^{g}, and Gm^{n} seems to be completely lacking in Negro populations, a possible crossing-over would not be detected unless very thorough family studies were performed including all the Gm(b) markers and Gm(x) which is probably extremely rare in Negroes.

The Gm^{n+} and the Gm^{n-} genes were found in connection with all of the following gene complexes of the γG1- and γG3-cistrons: Gm^{f} Gm^{b}, Gm^{f} Gm^{g}, Gm^{fa} Gm^{b}, Gm^{za} Gm^{g}, and probably Gm^{zax} Gm^{g}. The variation of Gm (n+) and Gm (n−) was much more frequent than those involving γG1- and γG3-markers. The findings were in accordance with family studies (7, 25) showing that the γG2-cistron is more distant from γG1 than is γG3. The Mongoloid Gm^{za} Gm^{b} was the only gene complex where this variability of (n+) and (n−) was not detected, although both antigens were present in the population. However, the Mongoloid Gm^{za} Gm^{b} complex is not frequent, and only a limited number of Gm^{za} Gm^{b}-homozygous individuals have been tested. Also, the Gm^{n} frequency is low in Japanese where most Mongoloid Gm^{za} Gm^{b} complexes were found.

Findings from the present family studies raise the possibility of a deletion of the γG3-cistron. The demonstration of low γG3-levels (very close to half the normal quantity) in the sera of persons that lacked both the γG3-genes, Gm^{b^0} and Gm^{g}, in one of their gene complexes favors this concept although the presence of a third allele is not excluded. Also a suppression of the product of one gene in a gene complex is possible, but is not very likely. This would involve inheritance of a suppressor property linked to the Gm system in several generations. The suppression would also have to be complete since very sensitive hemagglutination methods did not detect the gene product. A more likely explanation is a gene deletion. This could appear as a result of a chain-terminating mutation, or on the basis of an unequal crossing-over. Such crossing-over is known to play an important role in the development of proteins, the classical example being the Bar locus in *Drosophila melanogaster* (see references 29–31). In the human haptoglobin system unequal crossing-over has resulted in various gene duplications (30), and the opposite product of a gene duplication is a gene deletion. Furthermore, for the hemoglobins new genes and proteins originated from unequal nonhomologous crossing-over (31, 32). There are

also examples of probable gene deletions resulting in hereditary absence of enzymes and lack of serum proteins such as albumin, haptoglobin, transferrin, and complement (30, 33, 34). Such conditions behave as autosomal recessive traits, and the affected persons are homozygous for this trait. In some of these conditions it has been possible to determine the heterozygous state which is characterized by half the normal quantity of the respective proteins.

Further support was gained in the present study for the concept that variations of the gene complexes evolved through crossing-over. Recently recombina-

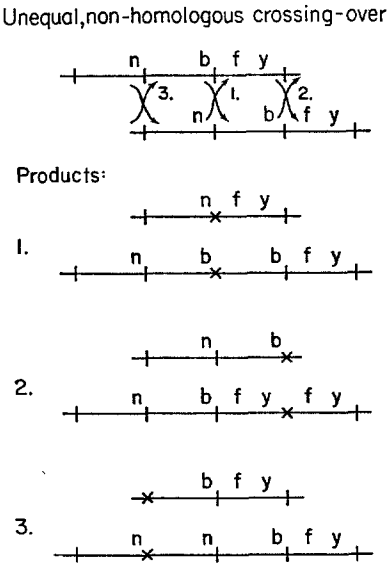

FIG. 4. Unequal nonhomologous crossing-over between two identical Gm gene complexes. The products are either a deletion or duplication of one of the three closely linked γG–heavy chain cistrons.

tion between cistrons was detected within families (7, 25). The detection of such crossing-over depends on the segregation of two different allelic genes at at least two cistrons. Thus the frequencies of the various genes in a given population were important. As mentioned, a crossing-over would not be detected in the Negro since probably nearly all individuals are $Gm^{za}\ Gm^{b}\ Gm^{n-}$ homozygous. Similarly, about 50% of Caucasians are homozygous for $Gm^{f}\ Gm^{b}\ Gm^{n}$, 50% of Chinese for $Gm^{fa}\ Gm^{b}\ Gm^{n}$, and nearly 50% of Japanese for $Gm^{za}\ Gm^{g}\ Gm^{n-}$. All such individuals are uninformative in showing recombination as a regular equal homologous crossing-over. In contrast, unequal crossing-over giving rise to gene deletions and duplications may be detected in homozygous individuals. Fig. 4 illustrates various types of unequal nonhomologous[3] crossing-

[3] The term nonhomologous has been used to designate crossing-over involving pairing of

over between two identical γG–heavy chain gene complexes, and the results which may be obtained, either deletions or duplications.

With a crossing-over in position 1 (Fig. 4) the product will either be a gene complex lacking Gm^b or a duplication complex with two Gm^b genes. The first product will give Gm^f,—,Gm^{n+}. Similarly, by crossing-over in other positions a deletion or a duplication at the γG1- and γG2-cistrons may appear. A possible γG1-gene deletion resulting in—,Gm^b Gm^{n+} or—,Gm^b, Gm^{n-} may therefore also be explained on this basis. Only the γG1- and γG3-subgroups can be tested at present by available Gm markers. A deletion of the γG2-cistron would not be detected because of the lack of a marker for the allele Gm^{n-}.

Gm^f Gm^b Gm^n is the most frequent gene complex in Caucasians, and the homozygous state is very common. The examples shown in Fig. 4 of unequal crossing-over would thus statistically be most frequent. This fits well with the existence of both γG3- and γG1-gene deletions that may originate from unequal nonhomologous crossing-over in Gm^f Gm^b Gm^n-homozygous persons. However, all different gene complexes may take part in such recombination events, giving rise to a variety of rare deletion complexes. Probably gene complexes with duplications of γG-cistrons will also be detected. Furthermore, products of nonhomologous crossing-over leading to new genes consisting of parts of two cistrons, should be looked for. Such a gene and its gene product is known from the Lepore hemoglobin (31, 32).

The presumed deletions add a further dimension to the marked heterogeneity of γG-gene complexes. The deletion complexes which probably developed through crossing-over, have since been transmitted as stable complexes in families. All the investigated γG3-deletions appeared with the same γG1- and γG2-markers, as Gm^f,—,Gm^{n+}. They may thus all have originated from the same genetic event although the families are now spread over different parts of Europe and in the United States. In the two families presented (Figs. 1 and 2) there was definitely not a fresh crossing-over, since more than one child carried the rare gene complex. In contrast, another family showed only two identical twins carrying the rare complex. They both had very low γG3-levels while the mother where the complex should originate had a much higher level of γG3. The possibility of a fresh unequal crossing-over in this mother exists.

The γ-globulin system is a striking example of allelic inactivation (Lyon effect) best known for the X-chromosome (35). Because of the "allelic exclusion" phenomenon which is characteristic of all γ-globulin–producing cells with formation of only one allelic gene product per cell, gene deletions in this system require special interpretation. The findings of low γG3-concentrations in the individuals with deletion gene complexes suggest that the allelic inactivation process and the mechanism of selection of one γG-subgroup cistron is not affected by the gene deletion. This would indicate that certain cells, although

independent cistrons, even though such pairing probably results from homologies between the cistrons.

having the genetic information for γ-globulin production, are nonresponsive because the single lacking gene is selected. It seems probable that certain of the quantitative variations in γ-globulin levels in disease may be due to deletions similar to those described in the present study, particularly if the homozygous state is encountered.

SUMMARY

The recently described Gm (g) and Gm (n) genetic markers of the γG3- and γG2-subgroups of γ-globulin were characterized in detail primarily through studies of myeloma proteins, their polypeptide chains and fragments. Antisera derived from rabbits, non-human primates and rheumatoid arthritis patients gave identical results. This contrasted with the Gm (b) system where the rabbit antisera react with a different genetic determinant (b^0) than the sera from rheumatoid arthritis patients (b^1). The Gm (g) and Gm (n) antigens were detected both by precipitin analysis and by hemagglutination inhibition. The Gm (g) antigen was not associated with any of the other genetic antigens of the γG3-proteins which all belonged in the Gm (b) class. The genes for the latter were always allelic to the gene coding for Gm (g), with that for Gm (b^0) constantly present when that for Gm (g) was absent.

The Gm (g) and Gm (n) markers were of particular value in tracing the various gene complexes made up of the closely linked subgroup genes. Further support was gained for the concept that the different gene complexes of various population groups arose primarily through crossing-over. The Gm^g and Gm^b genes for the γG3-subgroup were extremely closely linked to those for the γG1-subgroup. However the Gm (n) marker indicated that the γG2-subgroup genes were probably further separated on the chromosome. Additional evidence was obtained for the γG2-γG3-γG1–order of the subgroup cistrons.

Among the wide range of gene complexes a new type (γG2,—,γG1) was described. This complex appeared to have a deletion of the γG3-cistron. Lower levels of γG3-globulin were found in the sera of the individuals with this gene in the heterozygous state. The possibility that this unusual complex arose through an unequal nonhomologous crossing-over is discussed.

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