GM AND INV FACTORS IN SUBCLASSES OF HUMAN IgG*

By WILLIAM D. TERRY, M.D., JOHN L. FAHEY, M.D., AND ARTHUR G. STEINBERG, PH.D.

(From the Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, and the Department of Biology, Western Reserve University, Cleveland)

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Structural studies of IgG (7S γ_2-globulin) molecules indicate that they contain heavy and light polypeptide chains (1–5). Four antigenically different types of human IgG heavy chains (γ-chains) have been detected (6, 7) and are called γ_{2a}-, γ_{2b}-, γ_{2c}-, and γ_{2d}-chains.[1] Two antigenically distinguishable types of light chains, designated κ-and λ-chains, are also present in the normal IgG population (8, 9). About 65 per cent of normal serum IgG molecules contain κ-chains, 35 per cent contain λ-chains (8, 9), and these molecules are referred to respectively as type K and type L IgG. IgG molecules containing these types of heavy and light polypeptide chains are present in all normal sera.

Two loci, Gm (10) and Inv (11), determine a series of IgG antigens, or factors, which are present in the sera of some normal individuals, but not of others. This polymorphism of the human IgG population has been demonstrated by serological tests. Gm factors are associated only with heavy chains of IgG molecules (12–16) while Inv factors are associated with light chains of IgG, IgA, IgM, and also with Bence Jones proteins (13–16).

Gm and Inv factors have not been related to heavy and light polypeptide chain subclasses of normal serum IgG, because the four types of heavy chains and the two types of light chains are all present simultaneously in normal sera and methods for isolating each of the types from such sera are not now available. G myeloma (7S γ_2-myeloma) proteins, however, consist of molecules that are antigenically much more homogenous than those of normal serum IgG. G myeloma molecules have been shown to contain either κ- or λ-chains, but not both (17–19), and only one of the four types of γ-chains (6, 7). These proteins thus afford an opportunity to study relationships between Gm and Inv factors and antigenic subclasses of IgG heavy and light chains.

The distribution of three Gm factors [Gm(a), Gm(b), and Gm(f)][2] in myeloma proteins was studied by Kunkel *et al.* (20) who found that these factors were restricted to molecules in only two of the four γ-chain subclasses. γ_{2b}-Proteins (Group We) were either Gm(a+) or Gm(f+) and never Gm(b+). γ_{2c}-Proteins (Group Vi) were either Gm(b+) or Gm(−).

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[1] Alternative terminology for heavy-chain antigens: γ_{2a}, Ne; γ_{2b}, We; γ_{2c}, Vi; γ_{2d}, Ge (6).

[2] For comparison with Gm terminology used in this paper see Table I.

The present report contains an analysis of 89 G myeloma proteins, isolated from the sera of 1 Chinese, 60 white, and 28 Negro patients, that were classified according to γ-chain and light-chain antigens and tested for eight Gm factors [Gm(a), Gm(x), Gm(b^2), Gm(f), Gm(b^1), Gm(b^3), Gm(b^4), Gm(c)] and two Inv factors [Inv(l),[3] Inv(b)].[4] In addition, two "heavy-chain" disease proteins (21–23) were studied. Results were examined for relationships between the expression of these 10 genetically determined factors and the antigenic subclasses of heavy and light polypeptide chains.

Materials and Methods

Antisera.—(*a*) Antisera specific for human IgG, IgA, IgM, type K (type I), and type L (type II) determinants were produced in rabbits (24); and (*b*) γ_{2a}-, γ_{2b}-, γ_{2c}-, and γ_{2d}-antisera were obtained from rhesus monkeys repeatedly injected intramuscularly with either pooled normal human IgG, or isolated G myeloma proteins emulsified in complete Freund's adjuvant. All monkey antisera were absorbed with myeloma proteins of known γ-chain characteristics to render them specific for γ-chain subclass typing (7).

Immunodiffusion.—Immunoelectrophoresis and Ouchterlony analyses were performed with standard techniques using 1.5 per cent agar noble (Difco Laboratories, Inc., Detroit). Barbital buffer (pH 8.5, 0.045 M) was used for immunoelectrophoresis; Ouchterlony plates were prepared with 0.07 M NaCl, 0.03 M phosphate (pH 8) buffer.

Isolation and Characterization of G Myeloma Proteins.—Most proteins were isolated from either serum or plasma by zone electrophoresis (24), and several were prepared by DEAE-cellulose chromatography (24). Protein concentrations were calculated from optical density at 280 mμ using an extinction coefficient, $E_{1\ cm}^{1\ per\ cent}$ of 13.3. All isolated proteins were antigenically characterized and analyzed for purity with Ouchterlony tests. Each protein was examined at the highest available concentration (usually 10 to 30 mg/ml) and also at 1 mg/ml, with six antisera (four γ-chain antisera and two light-chain antisera). Tests at the lower concentration permitted characterization of the major component isolated, while those at higher concentrations yielded an estimation of the amount of contaminating IgG of other γ-chain and light-chain types. When indicated, the highest available concentration was also tested for the presence of IgA and IgM by Ouchterlony analysis using the appropriate specific antisera. All of the eight possible combinations of γ-chains and light chains have been identified (Table II), but type L γ_{2d}-protein was not available for isolation. "Heavy-chain" disease proteins Cr (from a Negro patient) and Zu (from a white patient) were gifts from Dr. E. C. Franklin and Dr. E. Osserman respectively.

Gm and Inv Typing.—Reagents used for typing the G myeloma and "heavy-chain" disease proteins are shown in Table I. All typing except for Gm(b^2) and Inv(b) was done on microflocculation slides as previously described (25). Tests for these two factors were done in 10 × 70 mm test tubes (26). Antibody Luc., used for detecting Inv(b), was a euglobulin precipitate prepared by dialysis against cold distilled water of a serum sample obtained from

[3] Inv(l) is a genetic factor that is present whenever Inv(a) is present, but that may also be found in the absence of Inv(a).

[4] The World Health Organization, Scientific Group on Genes, Genotypes and Allotypes of Immunoglobulins, has discussed a new notation for genetic factors of human immunoglobulins. The new notation substitutes numerical for alphabetical designations (Table I). As this notation is still under discussion, the present paper has used old designations and included proposed new designations only in tables of results.

Luc. 53 days after a transfusion. Although this serum lacked anti-Inv(b) activity, the euglobulin precipitate, dissolved in 0.1 M tris-HCl buffer pH 8, had activity identical with that of pretransfusion sera. All tests were done against group O R^1R^1 blood cells from the same donor. The cells were coated with the appropriate anti-D serum by incubating a mixture of one drop of washed packed RBC with two drops of anti-D and seven drops of saline at 37°C for 2 hours. Just before use, the coated cells were washed four times in normal saline and suspended at 0.3 per cent for the slide tests, which were read under a dissecting microscope, and at 1.5 per cent for the tube tests, which were read with the naked eye. All samples were tested at serial twofold dilutions. Initial protein concentrations were 2 mg/ml. Those samples scored as negative were negative at the first dilution (1 mg/ml). Inhibition titers are reported as the

TABLE I

Reagents Used to Detect 8 Gm and 2 Inv Factors

Genetic factor terminology		Genetic typing reagent dilutions	
Current	Suggested	Agglutinator	Anti-D*
Gm (a)	Gm (1)	Wils. 1/8	Rh 251
Gm (x)	Gm (2)	Tay. 1/32	Ham.
Gm (b^2)‡	Gm (3)	Da. 1/4	Roe.
Gm (f)§	Gm (4)	A. J. 1/8	"
Gm (b) and (b^1)	Gm (5)	Dr. 1/4	V. S.
Gm (b^3)	Gm (13)	Th. 1/4	"
Gm (b^4)	Gm (14)	Bu. 1/8	"
Gm (c)	Gm (6)	Ste. 1/2	War.
Inv (l)	Inv (1)	Math. 1/8	Roe.
Inv (b)‡	Inv (3)	Luc. euglobulin 1/8	Ham.

* All anti-D reagents used at 1/5 dilution.

‡ Tube tests; all others, slide tests.

§ Either very similar to or identical with Gm (b^2).

reciprocal of the last dilution (of a 2 mg/ml solution) giving a 2+ agglutination when the uninhibited control gave a 4+ agglutination (Appendix). Gm and Inv typing and assignment of phenotypes were done in Cleveland without knowledge of γ and light-chain types of the myeloma proteins which were determined in Bethesda. Similarly, γ and light-chain types were determined without knowledge of Gm and Inv types. Results of the Gm and Inv tests, and the phenotype assignments for all proteins are listed in the Appendix according to γ-chain and light-chain types and the race of the donor. Proteins 4, 28, 30, 32, and 90 could not be assigned definite phenotypes because of contamination of the myeloma protein with non-myeloma IgG, and these phenotypes are indicated as question marks.

RESULTS

Classification of G myeloma Proteins.—A total of 89 G myeloma proteins were isolated and analyzed for heavy and light chain antigenic characteristics (Table II). Every protein could be identified as belonging to one γ-chain subclass and as having light chains of one type. The frequency of γ-chain antigens was γ_{2a},

19 per cent; γ_{2b}, 67 per cent; γ_{2c}, 11 per cent; and γ_{2d}, 3 per cent. These proteins represent a selection, since all γ_{2a}-, γ_{2c}-, and γ_{2d}-myeloma sera available in sufficient quantity were fractionated, while only some γ_{2b}-myeloma sera were fractionated. A larger, unselected panel of 191 G myeloma sera (from which the 89 proteins were selected) contains 11 per cent γ_{2a}-, 77 per cent γ_{2b}-, 9 per cent γ_{2c}-, and 3 per cent γ_{2d}-myeloma proteins (Table II). The series of isolated proteins is therefore weighted in favor of the less commonly occurring subclasses. Absolute concentrations of the four subclasses of IgG in normal sera are not yet known, but preliminary quantitative studies indicate that the relative concentrations parallel the frequency with which the four subclasses occur in the unselected series (27).

TABLE II

γ-Chain and Light-Chain Types of G Myeloma Proteins and Serums

γ-Chain	Light-chain	89 Isolated G myeloma proteins	191 G myeloma serums
γ_{2a} (Ne)	κ*	9	11
	λ*	8	10
γ_{2b} (We)	κ	44	104
	λ	15	43
γ_{2c} (Vi)	κ	4	9
	λ	6	8
γ_{2d} (Ge)	κ	3	5
	λ	0	1

* κ light chains determine that a molecule is type K (type I); λ light chains determine that a molecule is type L (type II).

Light chain typing revealed that 67 per cent of the proteins were type K and 33 per cent type L (Table II). This 2:1 ratio is similar to the relative proportions of type K and type L reported in other collected series of G myeloma proteins (17–19). Of the four γ-chain subclasses, only γ_{2b}-proteins have an approximately 2:1 ratio of type K to type L. The γ_{2a}- and γ_{2c}-proteins have a 1:1 ratio of κ- to λ-chains (Table II). This 1:1 ratio may be due to the relatively small number of proteins tested, or it may indeed reflect an equal frequency of κ- and λ-chains associated with these γ-chain forms, in contrast to the 3:1 κ:λ ratio found with γ_{2b}-chains. The x^2 test comparing light-chain types of 147 γ_{2b}-proteins with light-chain types of 38 γ_{2a}- and γ_{2c}-proteins indicate that the observed differences in κ:λ ratios are unlikely to be due to chance alone ($0.05 > p > 0.02$). The very small series of γ_{2d}-myeloma proteins has a preponderance of type K molecules.

Correlations between Gm Factors and γ-Chain Subclasses.—

γ_{2a}- and γ_{2d}-Myeloma proteins: No Gm factors were detected in any of the 17 γ_{2a}- and three γ_{2d}-myeloma proteins (Tables III and IV, Appendix). Tests on two of the preparations (No. 30 and No. 32) showed low titers for several Gm

TABLE III

Gm and Inv Phenotypes of 89 G Myeloma Proteins Classified by γ and Light-Chain Types and by Race of Donor

γ-Chain	Light chain	Current		Suggested		No. of Samples		
		Gm	Inv	Gm	Inv	White	Negro	Chinese
γ_{2a} (Ne)	κ	—*	—	—	—	7	1	0
		?*	—	?	—	1	0	0
	λ	—	—	—	—	4	3	0
		?	?	?	?	0	1	0
γ_{2b} (We)	κ	b^2	b	3	3	13	0	0
		b^2	—	3	—	4	0	0
		b^2	l	3	1	1	1	0
		a	b	1	3	6	6	0
		a	l	1	1	0	4	0
		a	—	1	—	0	6	0
		ax	b	1, 2	3	1	0	0
		ax	—	1, 2	—	1	0	0
		?	?	?	?	0	1	0
	λ	b^2	—	3	—	5	0	0
		a	—	1	—	5	2	0
		ax	—	1, 2	—	2	0	0
		?	?	?	?	1	0	0
γ_{2c} (Vi)	κ	$b^{1, 3, 4}$	b	5, 13, 14	3	1	1	0
		$b^{1, 3, 4}$	—	5, 13, 14	—	1	0	0
		b^3	—	13	—	0	0	1
	λ	$b^{1, 3, 4}$	—	5, 13, 14	—	3	0	0
		$b^{1, 3, 4}$	?	5, 13, 14	?	1	0	0
		b^1c	—	5, 6	—	0	1	0
		$b^{1, 4}c$	—	5, 14, 6	—	0	1	0
γ_{2d} (Ge)	κ	—	—	—	—	3	0	0
Totals						60	28	1

* In all Tables: — indicates negative test result, ? indicates phenotype not assigned because of contamination.

factors and their Gm and Inv phenotypes are recorded as uncertain. Ouchterlony analysis of these preparations demonstrated molecules of heavy-chain subclasses other than γ_{2a}. The positive Gm results therefore are ascribed to contamination. While the γ_{2a}- and γ_{2d}-proteins lacked Gm antigens, all proteins of the other γ-chain subclasses contained at least one Gm antigen.

γ_{2b}-Myeloma proteins: Twenty-nine γ_{2b}-proteins were Gm(a+), 4 were Gm(a + x+), and 24 were Gm(b^2+) (Tables III and IV, Appendix). Two preparations (No. 4 and No. 90) were apparently Gm(a + b^2+), but these

TABLE IV

Summary of γ-Chains and Gm Phenotypes

γ-Chains	Gm phenotype		No. of samples		
	Current	Suggested	White	Negro	Chinese
γ_{2a} (Ne)	–	–	11	4	0
	?	?	1	1	0
γ_{2b} (We)	b^2	3	23	1	0
	a	1	11	18	0
	ax	1, 2	4	0	0
	?	?	1	1	0
γ_{2c} (Vi)	$b^{1, 3, 4}$	5, 13, 14	6	1	0
	b^1c	5, 6	0	1	0
	$b^{1, 4}c$	5, 14, 6	0	1	0
	b^3	13	0	0	1
γ_{2d} (Ge)	–	–	3	0	0
Totals			60	28	1

samples were shown to be heavily contaminated with non-myeloma IgG. Unfortunately, both donor patients are dead and no additional serum is available for refractionation. The phenotypes of protein samples 4 and 90 therefore could not be definitely established. If these proteins are excluded neither Gm(a) nor Gm(x) were found in Gm(b^2+) proteins. These findings confirm the observations of Kunkel *et al.* (20).

There was complete concordance between Gm(b^2) and Gm(f) reactions among the 87 myeloma proteins tested for both (51 of the γ_{2b}-myeloma proteins, and 26 other myeloma proteins). Tests for Gm(f) were not performed on 12 proteins because of reagent shortages. These results and those from tests of normal sera (28) suggest that Gm(b^2) and Gm(f) are very similar, if not identical genetic factors.

γ_{2c}-Myeloma proteins: Seven of 10 proteins were Gm($b^{1,3,4}$+). One γ_{2c}-protein (No. 88) was Gm(b^3+). This myeloma protein, obtained from the serum of the only Chinese patient available, provides further evidence for the existence in some Mongoloids of an allele determining Gm(b^3+) in the absence of Gm(b^1), Gm(b^2) and Gm(b^4) (29).

Gm(c) was detected only in γ_{2c}-myeloma proteins. One γ_{2c}-protein from a Negro patient (No. 63) was positive for factors Gm(b^1) and Gm(c), and another Negro myeloma protein (No. 71) was Gm($b^{1,4}$+ c+). Both phenotypes are of interest in that they establish for the first time a relationship between the genetic factors Gm(c) and γ_{2c}-heavy chains. The Gm($b^{1,4}$+ c+) protein is of special importance since it suggests the existence of a Gm allele previously undetected by studies of normal Negro sera (29).

"Heavy-chain" disease proteins: Two proteins, Cr and Zu, from patients with "heavy-chain" disease were tested for Gm and Inv factors. "Heavy-chain" disease proteins appear to be very similar, if not identical, to the Fc fragment that is obtained after papain digestion of IgG (21–23). Protein Zu has γ_{2c}-antigenic determinants and is Gm($b^{1,3,4}$+). The γ-chain antigenic determinants of protein Cr have not been clearly identified with monkey antisera. This protein, however, appears to be of type γ_{2d}. The Gm data are in accord with this impression since Cr was negative for the eight Gm factors. This finding differs from previous reports (21, 23) which described Cr as being Gm(b + e+). Tests for Gm(e) were not performed since reagents were not available for this factor. Previous detection of Gm(b) may have been due to contaminating IgG. As was expected, both "heavy-chain" disease proteins, which lack light chains, are Inv(l − b−).

Correlation Between Inv Factors and Myeloma Proteins with κ- (Type K) and λ- (Type L) Light Polypeptide Chains.—Inv factors were detectable only in type K protein preparations. Of 60 type K proteins, 6 were Inv(l+), 28 were Inv(b+), and 25 were Inv(l − b−). Inv(l + b+) proteins were not observed (Table V). None of the 29 type L proteins were definitely positive for Inv(l) or Inv(b). Three of these (Nos. 4, 32, and 90) could not be classified due to contamination with non-myeloma IgG. We emphasize that classification was done without knowledge of the light-chain type. Protein No. 28 was initially classified as Inv(b+), but was subsequently shown to contain non-myeloma IgG of type K.

Because of the low frequency with which Inv(l) was detected in the type K myeloma proteins (one of 39 from whites and 5 of 19 from Negroes), 65 of the sera from which these proteins were obtained were tested for Inv(l). Inv(l) was detected in 9 of 47 white sera (19 per cent) and 10 of 18 Negro sera (55 per cent). This does not differ from the expected frequencies of 20 per cent and 50 per cent Inv(l+) sera in normal white and Negro populations respectively (25). The low rate of occurrence of Inv(l+) type K myeloma proteins therefore

does not reflect an unusual genetic distribution in the myeloma patients from whom these proteins were obtained.

Correlations Between Inv Phenotype and γ-Chain Subclasses.—Of the 47 genetically classifiable type K γ_{2b}- and γ_{2c}-myeloma proteins, 34 were either Inv(1+) or Inv(b+) (Table III). By contrast, all of 12 classifiable type K γ_{2a}- and γ_{2d}-proteins were Inv(1 − b−). Although the number of proteins studied is relatively small, it is statistically unlikely that these 12 type K γ_{2a}- and γ_{2d}-proteins are Inv(1 − b−) due to chance alone ($p = 0.000005$).

TABLE V

Summary of Light Chains and Inv Phenotypes

Light chain	Inv phenotype		No. of samples		
	Current	Suggested	White	Negro	Chinese
κ	1	1	1	5	0
	b	3	21	7	0
	−	−	17	7	1
	?	?	0	1	0
λ	−	−	19	7	0
	?	?	2	1	0
Totals			60	28	1

DISCUSSION

These data confirm and extend the observations of Kunkel *et al.* (20) relating Gm factors (a), (b^1), and (f) to the four γ-polypeptide chain subclasses of G myeloma proteins. New information relating the Gm factors (b^2), (x), (b^3), (b^4), and (c) and the Inv factors (l) and (b) to the four γ-chain subclasses and to the two types of light polypeptide chains (κ and λ) is presented.

None of the eight Gm factors was detected in intact γ_{2a}- or γ_{2d}-myeloma proteins. Proteins of these γ-chain subclasses may contain as yet unrecognized genetic factors under the control of the Gm locus, or other known Gm factors, such as Gm(r), Gm(p), Gm(e), *etc.* that could not be assessed. Alternatively, γ_{2a}- and γ_{2d}-proteins may contain genetic factors controlled at a different locus, or they may not have any γ-chain genetic polymorphism. Another possibility is that γ_{2a}- and γ_{2d}-molecules may contain γ-chain genetic factors that are undetectable in the intact molecule because of tertiary or quaternary structural relationships. Serologic tests of γ-chains isolated from these molecules should be performed.

A single γ-polypeptide chain may contain multiple genetic factors. The ob-

servation of Gm(a + x+) γ_{2b}-proteins confirms reports (30–33) that both factors may be detected in a single myeloma protein. New examples of multiple factor expression are the γ_{2c}-proteins that are Gm($b^{1,3,4}$+) and the combinations of Gm($b^{1,4}$) and Gm(b^1) with Gm(c).

Certain facts concerning Gm factors and myeloma proteins can be interpreted in view of the data in this report. Three of the G myeloma proteins from Negroes (Nos. 62, 63, and 71) are Gm(b^1+). Although almost all American Negro sera are Gm(a + b^1+), Negro G myeloma proteins reported previously had been Gm(a+) and none had been Gm(b^1+). Previous failures to detect Gm(b^1+) proteins probably reflect the small numbers of myeloma proteins from Negroes that had been typed for Gm factors. The Gm(b^1) factor is expressed only in molecules containing γ_{2c}-chains. Only 9 per cent of G myeloma proteins are of subclass γ_{2c}, hence, less than 10 per cent of Negro G myeloma proteins would be expected to be Gm(b^1+).

It is of interest that myeloma proteins from Negro patients are phenotypically like those from white patients for the Gm(a), Gm(b^1), Gm(b^3), and Gm(b^4) factors. These factors, however, are inherited as a single unit in Negroes while Gm(a) on the one hand and Gm($b^{1,3,4}$) on the other are inherited as separate units in whites. This suggests that the expression of these factors is dependent on the type of γ-chain produced by the myeloma cells, as well as on the Gm genotype of the patient. The Gm genotype gives the cell the potential to produce one or more Gm factors, while the factors actually produced are dependent on the kind of γ-chain formed by the cell.

Gm(c) is detected only in Negro populations and is found in less than 30 per cent of sera in the American Negro population. Gm(c+) myeloma proteins, which also are members of the γ_{2c}-subclass, should therefore be detected even less frequently than Gm(b^1+) myeloma proteins. This proved to be the case with the finding of two Gm(c+) myeloma proteins.

A clear relationship has been demonstrated between γ_{2b}-myeloma proteins (polypeptide chains) and Gm factors (a), (x), (b^2), and (f) and between γ_{2c}-myeloma proteins (polypeptide chains) and Gm factors (b^1), (b^3), (b^4), and (c). There are various interpretations of how the observed relationships between Gm factors and different forms of γ-polypeptide chains might influence theoretical conceptions of the Gm locus. Discussion of these concepts is outside the scope of this report, and a more complete exposition of the problem has been presented elsewhere (20, 28).

Inv factors were detected only in type K myeloma proteins. All κ-polypeptide chains must share a common amino acid sequence which determines the κ-specific configuration. In addition, they must contain highly variable segments which are responsible for individual specific configurations (34, 35). Presumably, Inv factors are present in the common segment. Approximately 40 per cent of the type K proteins were Inv(1 − b−). Light chains of these proteins may con-

tain Inv factors that have not yet been recognized or defined. Another possibility is that these light chains represent a subclass of κ-chains under the genetic control of another locus.

All of the type L myeloma proteins that could be unequivocally classified were Inv(1 − b−). Three instances of type L G myeloma proteins containing Inv factors have been reported (13, 31, 36). Inadequacies in some aliquots of the Inv(b) agglutinator serum (Luc.), however, may have contributed to these findings. The recent report of Lawler and Cohen (36) stated that the isolated λ-chains of one G myeloma protein were Inv(b+). The anti-Inv(b) serum used in these tests was obtained from Luc. shortly after a transfusion, and was subsequently noted to give unreliable test results. Through the kind cooperation of Dr. Lawler and Dr. Cohen, one of us (A.G.S.) retested these λ-chains with an aliquot of reliable anti-Inv(b) and the chains were clearly Inv(b−). In view of this and the data in the present study, it seems unlikely that type L G myeloma proteins contain Inv factors detectable in the intact molecule. Light chains isolated from Inv(1 − b−) type L G myeloma proteins may possibly contain Inv factors that are not detectable when those light chains are folded into the intact IgG molecule. This also is not likely, since λ–Bence Jones proteins, which appear to be very similar to light chains isolated from myeloma proteins, have almost all been Inv(1 − b−) (13, 31, 37).

Gm and Inv factors are controlled by independent genetic loci. Because the loci are independent, it might be expected that the occurrence of Gm and Inv factors in a single IgG molecule would also be an independent phenomenon, *i.e.*, that the occurrence of Inv factors in a molecule would be independent of the occurrence of Gm factors. The current data showing that type K γ_{2a}- and γ_{2d}-proteins which lack Gm antigens are also Inv(1 − b−) cast some doubt on this assumption. This finding may be due to genetic control in the sense that cells synthesizing γ_{2a}- or γ_{2d}-heavy chains might not be able to synthesize κ-chains expressing Inv factors. Alternatively, the conformation of κ-chains when folded with γ_{2a}- or γ_{2d}-chains may be such that Inv determinants are blocked or distorted.

All the data in the present report are derived from studies of G myeloma proteins. Proof that the observed relationships are also true for normal serum IgG must await isolation of γ_{2a}-, γ_{2b}-, γ_{2c}, and γ_{2d}-globulins and κ- and λ-chains from normal serum.

SUMMARY

Human G myeloma (7S γ_2-myeloma) proteins were investigated for relationships between Gm and Inv genetic factors and the different antigenic types of heavy polypeptide chains (γ-chains) and light polypeptide chains.

Myeloma proteins were isolated from the sera of 1 Chinese, 60 white and 28 Negro individuals. These 89 proteins were tested for eight Gm factors [Gm(a),

Gm(x), Gm(b^2), Gm(f), Gm(b^1), Gm(b^3), Gm(b^4), and Gm(c)], and two Inv factors [Inv(l) and Inv(b)]. Results of the tests were correlated with the four γ-chain subclasses (γ_{2a}, γ_{2b}, γ_{2c}, and γ_{2d}) and the two types of light polypeptide chains, κ-chains (type K or I) and λ-chains (type L or II) found in human IgG molecules.

1. Gm factors were limited to myeloma proteins with heavy polypeptide chains of the γ_{2b}- and γ_{2c}-subclasses. No Gm factors were detected on γ_{2a}- and γ_{2d}-myeloma proteins or on a "heavy-chain" disease protein of subclass γ_{2d}.

2. γ_{2b}-Proteins were positive for at least one Gm factor and were either Gm(a+), Gm(a + x+), or Gm(b^2+ f+).

3. γ_{2c}-Myeloma proteins, and one γ_{2c}-"heavy-chain" disease protein, were positive for at least one Gm factor and contained various combinations of factors Gm(b^1), (b^3), (b^4), and (c). Myeloma proteins from 3 Negroes were included in this group.

4. Inv factors (l) and (b) were limited to myeloma proteins with κ-light polypeptide chains. These Inv factors were not detected on proteins with λ-light polypeptide chains.

5. Most (70 per cent) of the γ_{2b}- and γ_{2c}-proteins with κ-chains were Inv(l+) or Inv(b+). None of the γ_{2a}- or γ_{2d}-proteins with κ-chains, however, contained these Inv factors.

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Note Added in Proof.—Another myeloma protein, from Chinese patient W.P. (No. 92) has recently been studied in our laboratories. The serum phenotype is Gm (a+, b^2+, b^1+, b^3+, b^4+, x−), Inv(l − b+). The isolated myeloma protein is of subclass γ_{2b}, type K. Titration data for Gm factors are: Gm(a), 256; Gm(b^2), 256; Gm(b^1), −; Gm(b^3) −; Gm(b^4) −. The phenotype of this mongoloid myeloma protein is clearly Gm(a + b^2 +). A similar finding, based on titration of a mongoloid myeloma serum, has recently been reported (38). This combination of Gm factors in a myeloma protein indicates that the genetic information for these factors may be carried on the same chromosome and raises the question of why Gm(a + b^2 +) molecules are not observed in myeloma proteins from Caucasoids.

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See following page for Appendix

APPENDIX

Gm and Inv Factors of Human G Myeloma Proteins

γ-Chain	Light-chain	Race	Sample No.	Gm								Inv		Phenotype			
				New (1)	(2)	(3)	(4)	(5)	(13)	(14)	(6)	New (1)	(3)	Gm		Inv	
				Old (a)	(x)	(b^2)	(f)	(b^1)	(b^3)	(b^4)	(c)*	Old (l)	(b)	Old	New	Old	New
γ_{2a} (Ne)	κ(I)	Caucasoid	5	—‡	—	16	8	—	—	—		—	—	—	—	—	—
			6	—	—	—	—	—	—	—		—	—	—	—	—	—
			8	—	—	—	—	—	—	—		—	—	—	—	—	—
			10	—	—	16	4	—	—	—		—	—	—	—	—	—
			27	—	—	—	—	—	—	—		—	—	—	—	—	—
			30	16	—	32	32	—	8	—		—	16	?‡	?	?	?
			31	8	—	—	—	—	—	—		—	2	—	—	—	—
			84	8	—	—	NT‡	—	4	—		—	—	—	—	—	—
		Negroid	76	—	—	—	—	2	2	—	—	—	—	—	—	—	—
	λ(II)	Caucasoid	7	—	—	—	—	—	—	—		—	—	—	—	—	—
			9	—	—	—	—	—	—	—		—	—	—	—	—	—
			11	—	—	4	4	—	—	—		—	—	—	—	—	—
			26	—	—	—	—	—	—	—		—	—	—	—	—	—
		Negroid	32	64	—	—	—	8	8	—	—	—	32	?	?	?	?
			66	16	32	—	—	—	32	—	—	—	—	—	—	—	—
			72	8	—	—	—	4	2	—	—	8	—	—	—	—	—
			73	8	—	—	—	2	2	—	—	2	2	—	—	—	—
γ_{2b} (We)	κ(I)	Caucasoid	1	8	16	64	256	—	—	—		—	128	b^2	3	b	3
			2	—	—	128	256	—	—	—		—	—	b^2	3	—	—
			13	256	256	—	—	—	4	—		—	128	ax	1 ,2	b	3
			14	—	—	512	512	—	—	—		—	—	b^2	3	—	—
			15	—	—	256	512	16	8	4		—	—	b^2	3	—	—
			18	2	—	512	512	64	64	16		—	256	b^2	3	b	3
			21	64	32	256	128	16	64	—		—	256	b^2	3	b	3
			29	—	—	128	256	—	—	—		—	256	b^2	3	b	3
			35	128	—	—	—	—	—	—		—	128	a	1	b	3
			36	—	—	256	256	64	64	64		—	128	b^2	3	b	3
			38	—	—	128	256	—	—	—		—	—	b^2	3	—	—
			39	128	—	—	—	—	—	—		—	32	a	1	b	3
			41	—	—	256	128	—	4	—		—	64	b^2	3	b	3

		42	16	—	256	128	16	—	—		—	64	b^2	3	b	3
		45	—	—	512	128	—	—	—		—	32	b^2	3	b	3
		51	—	—	256	128	—	—	—		—	64	b^2	3	b	3
		52	256	—	8	2	—	—	—		—	32	a	1	b	3
		53	128	—	8	4	—	—	—		—	32	a	1	b	3
		54	—	—	256	256	—	—	—		—	64	b^2	3	b	3
		59	—	—	256	256	—	—	—		512	—	b^2	3	l	1
		67	—	—	512	128	—	8	—		—	512	b^2	3	b	3
		68	16	—	256	128	16	32	16		—	64	b^2	3	b	3
		69	256	8	—	—	—	—	—		—	64	a	1	b	3
		70	—	—	512	256	—	16	—		—	128	b^2	3	b	3
		80	4096	—	8	NT	—	16	2		—	512	a	1	b	3
		83	512	1024	8	NT	—	4	—		—	—	ax	1, 2	—	—
	Negroid	16	512	—	—	—	—	—	—	—	—	—	a	1	—	—
		23	—	—	256	256	—	—	—	—	1024	—	b^2	3	l	1
		33	64	—	—	—	—	—	—	—	—	512	a	1	b	3
		46	256	—	16	32	16	8	—	—	—	32	a	1	b	3
		50	64	—	—	—	—	4	—	8	—	32	a	1	b	3
		56	256	—	—	—	—	—	—	—	—	16	a	1	?	?
		57	128	—	—	—	—	—	—	—	2	32	a	1	b	3
		64	128	—	—	—	—	—	—	—	—	32	a	1	b	3
		74	32	—	—	—	—	2	—	—	—	2	a	1	—	—
		75	64	—	—	—	16	16	2	—	256	—	a	1	l	1
		77	64	—	—	—	8	4	—	16	128	—	a	1	l	1
		82	128	—	—	NT	2	4	—	—	128	—	a	1	l	1
		85	256	—	—	NT	4	8	—	—	—	—	a	1	—	—
		86	256	—	8	NT	8	16	2	—	—	—	a	1	—	—
		87	256	—	—	NT	—	16	—	—	—	2	a	1	—	—
		90	128	—	128	NT	16	16	8	—	128	4	?	?	?	?
		91	256	—	—	NT	4	16	2	—	16	—	a	1	—	—
		78	32	—	—	—	4	4	—	—	64	4	a	1	l	1
λ(II)	Caucasoid	3	128	128	4	8	—	—	—		—	—	ax	1, 2	—	—
		4	64	—	64	64	8	8	4		—	32	?	?	?	?
		17	—	—	256	512	—	—	—		—	—	b^2	3	—	—
		19	—	—	32	32	—	—	—		—	—	b^2	3	—	—
		37	512	—	—	—	—	—	—		—	—	a	1	—	—
		43	—	—	256	256	—	—	—		—	—	b^2	3	—	—

(*Appendix continued on following page*)

APPENDIX—*Concluded*

Gm *and Inv Factors of Human G MyelomaProteins*

γ-Chain	Light-chain	Race	Sample No.	Gm								Inv		Phenotype			
				New (1)	(2)	(3)	(4)	(5)	(13)	(14)	(6)	New (1)	(3)	Gm		Inv	
				Old (a)	(x)	(b^2)	(f)	(b^1)	(b^3)	(b^4)	(c)*	Old (1)	(b)	Old	New	Old	New
γ_{2b}(We)	λ(II)	Caucasoid	44	512	—	—	—	—	—	—		—	—	a	1	—	—
			47	64	—	—	—	—	2	—		—	—	a	1	—	—
			48	256	—	8	8	4	4	—		—	—	a	1	—	—
			49	—	—	512	256	—	—	—		—	2	b^2	3	—	—
			55	128	128	16	8	—	—	—		—	4	ax	1, 2	—	—
			58	—	—	256	256	—	—	—		—	—	b^2	3	—	—
			61	64	—	—	—	—	—	—		—	—	a	1	—	—
		Negroid	60	128	—	—	—	—	—	—	—	—	—	a	1	—	—
			65	1024	—	—	—	—	—	—	8	—	—	a	1	—	—
γ_{2c} (Vi)	κ(I)	Caucasoid	25	—	—	8	4	128	128	64		—	128	$b^{1,3,4}$	5, 13, 14	b	3
			81	8	—	4	NT	1024	1024	2048		16	—	$b^{1,3,4}$	5, 13, 14	—	—
		Negroid	62	8	—	—	—	256	256	256	—	—	32	$b^{1,3,4}$	5, 13, 14	b	3
		Mongoloid	88	8	—	—	NT	—	64	—	—	—	4	b^3	13	—	—
	λ(II)	Caucasoid	20	—	—	—	—	256	512	256		—	—	$b^{1,3,4}$	5, 13, 14	—	—
			24	8	—	16	32	512	512	512		—	8	$b^{1,3,4}$	5, 13, 14	—	—
			28	—	—	32	64	256	128	128		—	64	$b^{1,3,4}$	5, 13, 14	b?	3?
			34	—	—	—	—	128	128	64		—	—	$b^{1,3,4}$	5, 13, 14	—	—
		Negroid	63	—	—	—	—	512	—	16	512	—	—	b^1, c	5, 6	—	—
			71	8	—	—	—	512	16	512	512	—	4	$b^{1,4}$, c	5, 14, 6	—	—
γ_{2d} (Ge)	κ(I)	Caucasoid	22	—	—	—	—	—	—	—		—	—	—	—	—	—
			40	—	—	—	—	—	—	—		—	16	—	—	—	—
			89	—	—	—	NT	—	—	—		—	—	—	—	—	—

* Caucasoid myeloma proteins not tested for Gm(c) [Gm(6)].

‡? indicates phenotype not assigned; — indicates negative test;NT indicates not tested.