Comparison of Gm(f) with Gm(b^2) [Gm(b^w)] and a Discussion of their Genetics

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THERE HAVE BEEN at least three reports of a genetic determinant of gamma globulin called Gm(f), which the authors of the reports claim is a new factor (Gold *et al.*, 1965; Kunkel *et al.*, 1964; and Mårtensson, 1964). Studies of this factor have led to the postulation of additional loci (i.e., additional to the *Gm* locus) for the control of the Gm factors of 7S gamma globulin (Kunkel *et al.*, 1964; Mårtensson, 1964).

Certain characteristics of this determinant have led me to compare it with Gm(b^2) (Steinberg and Goldblum, 1965) [originally called Gm(b^w) (Steinberg and Wilson, 1963)]. This paper is a report of the comparison of Gm(f) and Gm(b^2) and a discussion of their genetics.

MATERIALS AND METHODS

Sera. Two hundred fifty-five serum samples (from U. S. Negroes and whites; Brazilians of mixed white, Negro, and South American Indian ancestry; Bushmen and Bantu from South Africa; and Melanesians from Papua, New Guinea) which had been previously typed for Gm(a), Gm(x), Gm(c), and four Gm (b) antigens (Steinberg and Goldblum, 1965) were selected for typing with anti-Gm(f) sera. (The anti-Gm(f) sera were generously supplied by Drs. E. R. Gold, Rune Grubb, and Claude Ropartz.) I am indebted to Drs. Newton Morton, Trefor Jenkins, and Eugene Giles for some of the serum samples used in these experiments.

Reagents. The reagents used to determine the Gm types except Gm(f) are listed in Table 1 of the paper by Steinberg and Goldblum (1965). The method of conducting the tests has been previously described (Steinberg, 1962). Gm(f) was determined with the anti-Gm(f) antibodies A.J. and Lister; Gm(b^2) was determined, as previously reported, with anti-Gm(b^2) Da (Steinberg and Wilson, 1963). The anti-Gm(f) and anti-Gm(b^2) antibodies were used against group O R^1R^1 cells coated 1/5 with anti-D Roehm.

THE DATA

A summary of the 255 comparisons arranged by the race and Gm types of the donors is presented in Table 1. All 255 comparisons were concordant;

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TABLE 1. SUMMARY OF THE COMPARISONS OF Gm(b^2) WITH Gm(f)*

A. American Negroes

No.	a	x	c	b^1	b^3	b^4	b^2	f
8	+	−	+	+	+	+	−	−
4	+	−	+	+	−	−	−	−
4	+	−	−	+	+	+	−	−
1	+	−	−	+	+	−	−	−
6	+	−	+	+	+	+	+	+
12	+	−	−	+	+	+	+	+
7	−	−	−	+	+	+	+	+

$b^2(+)$, $f(+) = 25$; $b^2(-)$, $f(-) = 17$.

B. American whites

No.	a	x	c	b^1	b^3	b^4	b^2	f
3	+	+	−	−	−	−	−	−
12	+	−	−	−	−	−	−	−
7	+	−	−	+	+	+	+	+
3	+	+	−	+	+	+	+	+
4	−	−	−	+	+	+	+	+
2	+	−	−	−	−	−	+	+

$b^2(+)$, $f(+) = 16$; $b^2(-)$, $f(-) = 15$.

C. Brazilians of mixed white, Negro, and South American Indian ancestry

No.	a	x	c	b^1	b^3	b^4	b^2	f
7	+	−	−	−	−	−	−	−
1	+	+	−	−	−	−	−	−
1	+	−	+	+	−	−	−	−
6	+	−	−	+	+	+	+	+
2	−	−	−	+	+	+	+	+
1	+	+	−	+	+	+	+	+
3	+	−	+	+	−	−	+	+
7	+	−	−	−	+	−	+	+

$b^2(+)$, $f(+) = 19$; $b^2(-)$, $f(-) = 9$.

D. South Africans
(1) Bushmen

No.	a	x	c	b^1	b^3	b^4	b^2	f
19	+	−	−	−	+	−	−	−
19	+	−	−	+	+	+	−	−
2	+	−	−	+	−	−	−	−
1	+	−	−	+	+	−	−	−
2	+	−	+	+	+	+	−	−
1	+	−	+	+	−	−	−	−
1	+	−	+	+	−	+	−	−

$b^2(+)$, $f(+) = 0$; $b^2(-)$, $f(-) = 45$.

D. South Africans
(2) Bantu

No.	a	x	c	b^1	b^3	b^4	b^2	f
1	+	−	−	+	+	+	−	−
1	+	−	+	+	+	+	−	−
1	+	−	+	+	−	+	−	−
2	+	−	+	+	+	−	−	−

$b^2(+)$, $f(+) = 0$; $b^2(-)$, $f(-) = 5$.

TABLE 1. (CONTINUED)

No.	a	x	c	b^1	b^3	b^4	b^2	f
E. New Guinea (Papua) natives								
4	+	−	−	−	−	−	−	−
25	+	−	−	+	+	+	−	−
75	+	−	−	+	+	+	+	+
$b^2(+)$, f(+) = 75; $b^2(-)$, f(−) = 29.								
Total: $b^2(+)$, f(+) = 135; $b^2(-)$, f(−) = 120.								

*The generic name of the locus (*Gm*) is omitted from the table.

135 were positive for Gm(b^2) and Gm(f), and 120 were negative for Gm(b^2) and Gm(f). The conclusion that Gm(b^2) and Gm(f) are the same, at least in our hands,* seems warranted and is reinforced by the observations that (1) these antigens are the only Gm antigens found on the S fragment of 7S gamma globulin obtained with either papain or pepsin digestion of gamma globulin; (2) each may be detected only when the H and L chains are combined and not on either alone; and (3) the information for each is carried by the H chain and not the L chain (Steinberg and Polmar, 1965; Polmar and Steinberg, unpublished).

Since the two antigens probably are the same, they henceforth will be referred to as Gm(b^2) or Gm(f) interchangeably, although Gm(b^2) is preferred.

DISCUSSION

Kunkel *et al.* (1964) have suggested on the basis of immunological data that two closely linked loci, called *We* and *Vi,* and possibly three or more, determine the Gm factors. They state from their results that, ". . . it seems possible that Gm^f (certainly not Gm^b) may be the true allele of Gm^a" and that, "It is then a reasonable concept that the 'We locus' (with Gm^a and possibly Gm^f as major alleles) and the 'Vi locus' (with Gm^b and at least one unknown allele) are closely linked." They state that, "Reasoning along these lines leads to the prediction that Gm(b−) γ-globulins of the Vi group contain the product of the true allele(s) of Gm^b and that genes at a third locus, the 'Ge locus,' as well as other loci, determine characteristics of the H chains of other 7S γ-globulin molecules."

Since in Caucasoids Gm(f) is always associated with Gm(b^1), it follows that the Gm^f allele of the postulated *We* locus is always in the *cis* position relative to the $Gm^{b(1)}$ allele of the *Vi* locus and that the Gm^a allele of the *We* locus is always in the *cis* position relative to the undetected allele(s) of the *Vi* locus (see Fig. 1).

Since Gm(f) probably represents a rediscovery of Gm(b^2), or something very like it, and since considerable family and population data concerning

*Gold (personal communication) reports that he and his colleagues have found three Hawaiian sera which are Gm(b^2+f−).

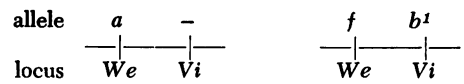

FIG. 1. Diagram showing the associations in Caucasoids of the alleles at the *We* and *Vi* loci postulated by Kunkel *et al.*, 1964. The distance between the loci is greatly exaggerated.

the genetics of Gm(b^2) and other Gm antigens are available (Steinberg and Wilson, 1963; Steinberg and Goldblum, 1965), it is appropriate to use these data to evaluate the likelihood that more than one locus determines the Gm factors. "Locus," as used in this paper, means a series of nucleotide pairs (certainly some hundreds and possibly thousands) which carry the information for the synthesis of a single molecule (i.e., polypeptide chain) and within which mutations (changes in one or more nucleotide pairs) and crossing over may occur.

Four different antisera have been shown, by tests of more than 100 serum samples from white donors, to detect Gm(b) in Caucasoids. Tests of these antibodies against serum samples from American Negroes, African Negroes, Japanese, Chinese, North American Indians, Eskimos, Australian aborigines, Melanesians from New Guinea, and from Brazilian families of mixed white, Negro, and Indian ancestry established that these four antibodies detect four different antigens (Steinberg and Goldblum, 1965). The antigens have been named Gm(b^1), Gm(b^2), Gm(b^3), and Gm(b^4). Gm(b^1) corresponds to the Gm(b) factor reported by Harboe (1959), Gm(b^2) corresponds to Gm(b^w), while Gm(b^3) and Gm(b^4) are new.

The Gm^b allele of whites and the Gm^{ab} allele of Mongoloids determine all four of these antigens. None of these antigens is determined by the Gm^a or Gm^{ax} alleles of whites or by the Gm^{ax} allele of Mongoloids. Eleven of the 16 possible combinations of these four antigens have been observed. The nine combinations observed in addition to the two mentioned above (all four present and all four absent) are determined by various alleles (some rare) in the several races examined. A summary of the reactions of the various Gm alleles (alleles as used here refers to the original usage of the Gm factors) with antibodies to the four Gm(b) antigens is presented in Table 2.

Mongoloids are all Gm(a+). The frequency of Gm(b^1+) individuals among the Mongoloids varies from population to population (see Ropartz, Rousseau, and Rivat, 1961, for a summary of earlier data; Steinberg and Matsumoto, 1964). Thus far, all sera from Mongoloids which have been Gm(b^1+) have also been Gm(b^2+), Gm(b^3+), and Gm(b^4+) (Table 2). Those which have been Gm(b^1–) were also always Gm(b^2–) and Gm(b^4–) (Table 2), but about 50% of the samples which were Gm(a+b^1–x–) were Gm(b^3+) (Table 2).

Our findings for Gm(b^2) in Caucasoids and Mongoloids have been confirmed by Gold *et al.* (1964).

If Gm(b^2) is determined by an allele of Gm(a), and Gm(b^1) is determined by an allele at a second locus, one would expect to find among Caucasoids and Mongoloids a chromosome determining Gm(a) and Gm(b^1)

TABLE 2. REACTIONS OF THE MORE COMMON ALLELES IN NEGROIDS, MONGOLOIDS, AND CAUCASOIDS WITH THE GM(B) ANTIBODIES AND A SUGGESTED NOMENCLATURE*

Race	Allele: Current terminology	Allele: Suggested terminology	Antibody: Draves Antigen detected: Gm(b^1)	Da Gm(b^2)	Th Gm(b^3)	Bu Gm(b^4)
Negroid	Gm^{ab}	$Gm^{ab(1,3,4)}$	+	−	+	+
	Gm^{ab}	$Gm^{ab(1,4)}$	+	−	−	+
	Gm^{abc}	$Gm^{ab(1)c}$	+	−	−	−
Mongoloid	Gm^{a}	Gm^{a}	−	−	−	−
	Gm^{a}	$Gm^{ab(3)}$	−	−	+	−
	Gm^{ax}	Gm^{ax}	−	−	−	−
	Gm^{ab}	$Gm^{ab(1,2,3,4)}$	+	+	+	+
Caucasoid	Gm^{a}	Gm^{a}	−	−	−	−
	Gm^{ax}	Gm^{ax}	−	−	−	−
	Gm^{b}	$Gm^{b(1,2,3,4)}$	+	+	+	+

*This table is identical with Table 11 of the paper by Steinberg and Goldblum (1965).

but not Gm(b^2). This follows from the demonstration that in a population at equilibrium the frequency with which alleles at different loci are associated with one another in the same gamete is proportional to the frequency of the alleles in the population (Robbins, 1918). This relationship holds whether or not the genes are linked. Linkage merely affects the approach to equilibrium.*

The combined frequency of the Gm^a and Gm^{ax} alleles in U. S. whites is approximately .30, and the frequency of the allele determining the Gm(b) antigens is .70 (Steinberg *et al.*, 1961). Since the latter allele determines Gm(b^1), we would expect to find in whites at equilibruim a chromosome determining Gm(a) and Gm(b^1) with a frequency equal to .3 × .7, or .21. If the population is not in equilibrium, the frequency of the chromosome will be less than .21. How much less depends upon how many generations have transpired since the origin of these factors and how closely they are linked. Since the expected chromosome has not been observed, we must conclude either (1) that the population has only recently acquired these antigens, that they were acquired in the *trans* position, and that recombination (at detectable frequencies) has not yet occurred or (2) that the hypothesis that Gm(a) and Gm(b^1) are determined by genes at different loci is incorrect. It is unlikely that the population has only recently acquired Gm(a) and Gm(b^1), because Boyer and Young (1961) have shown that Gm(a) and Gm(b) [probably Gm(b^1)] occur in chimpanzees.

The hypothesis that two or more loci determine the Gm factors encounters further difficulty when the occurrence of the Gm(b) antigens is considered

*The approach to equilibrium is described by the expression $\triangle_n = \triangle_0(1-c)^n$, where $\triangle_n$ equals the difference between the frequencies in generation n and those expected at equilibrium, $\triangle_0$ equals the difference at the initiation of the approach to equilibrium, and c equals the crossover frequency between the loci in question (Robbins, 1918).

among races other than Caucasoids. In Mongoloids, as in Caucasoids, alleles which produce Gm(b^1) also produce Gm(b^2), Gm(b^3), and Gm(b^4) (Steinberg and Goldblum, 1965); however, all Mongoloids are Gm(a+) (Ropartz, Rivat, Rousseau, and Lenoir, 1961; Steinberg and Matsumoto, 1964). Hence, in Mongoloids the same chromosome carries determinants for Gm(a) and Gm(b^2). Therefore, the assumption that alternative alleles determine Gm(a) and Gm(b^2) cannot be valid for Mongoloids. Mårtensson (1964) recognized this and wrote that the observations on Chinese and Japanese ". . . would obviously preclude (at least for certain populations) the hypothesis that the specificities Gm(a) and Gm(f) [i.e., Gm(b^2)] or Gm(a) and Gm(b) [i.e., Gm(b^1)] are elaborated by genes behaving as alternate alleles."

Mårtensson does not state how he believes these factors are inherited. To retain the two-locus hypothesis, it would be necessary to assume that the *We* locus in Mongoloids has a $Gm^{ab(2)}$ (Gm^{af}) allele and that this allele is always in the *cis* position with the $Gm^{b(1)}$ allele of the *Vi* locus, while the Gm^{a} and Gm^{ax} alleles of the *We* locus are always in the *cis* position with the undetected allele of the *Vi* locus. Even with this assumption, the multiple-locus hypothesis still leaves unexplained why in both Mongoloids and Caucasoids the same chromosome always produces Gm(b^2) and Gm(b^1), regardless of the presence (in Mongoloids) or absence (in Caucasoids) of Gm(a).

Negroids do not as a rule have the Gm(b^2) antigen (Steinberg and Wilson, 1963; Steinberg and Goldblum, 1965), but all are Gm(a+b^1+). The interpretation of these data on the multiple-locus hypothesis requires that in this race the Gm^{a} allele of the *We* locus is always in the *cis* position relative to the $Gm^{b(1)}$ allele of the *Vi* locus.

Further complications arise for the multiple-locus hypothesis when the antigens Gm(b^3) and Gm(b^4) are considered among the non-Caucasoid races (Table 2).

In Negroids the usual Gm^{abc} allele produces only the Gm(b^1) antigen, in addition to Gm(a) and Gm(c). Some Gm^{ab} alleles, on the other hand, produce Gm(b^3) and Gm(b^4) in addition to Gm(a) and Gm(b^1), while others fail to produce Gm(b^3) (Table 2). We have observed Gm(b^2) associated with the Gm^{ab} (Steinberg and Wilson, 1963) and with the Gm^{abc} alleles (Steinberg and Goldblum, 1965) of Negroes, albeit rarely.

The Gm(b) antigens occur in the presence of Gm(a) in Mongoloids and Negroids (Table 2). An explanation for the occurrence of Gm(a), Gm(b^1), and Gm(b^2) in the same gamete, based on the multiple-locus hypothesis, was offered above. On the basis of this hypothesis, it seems necessary to invoke two more loci to account for the gametic distribution of Gm(b^3) and Gm(b^4), since each may occur in the presence or absence of the other, or of Gm(b^2), or of Gm(b^1), although Gm(b^4) rarely occurs in the absence of Gm(b^1). If there are indeed four loci, the almost exclusive occurrence of all four alleles determining Gm(b) antigens in the *cis* position in Caucasoids and their very common occurrence in the *cis* position in Mongoloids, combined with the failure of any two to approach random distribution, is indeed astonishing. There is still another observation which is incompatible with

the multiple-locus hypothesis. None of the Gm(b) antigens is determined by the same "chromosome" which determines both Gm(x) and Gm(a), yet in Mongoloids the Gm(b) antigens are determined by the same chromosome which determines Gm(a) (Table 2). It is odd, on the basis of the multiple-locus hypothesis, that the Gm^{ax} allele should always occur in the *trans* position relative to the Gm^{b} alleles.

It may be correctly argued that Kunkel *et al.* (1964) postulated multiple loci on the basis of immunological data and not on the basis of family or population data and that their observations remain to be explained. In brief, Kunkel *et al.* found that 7S γ-globulin myeloma proteins may be divided into four groups on the basis of three antisera against 7S γ-H chains. All Gm(b^1+) myeloma proteins were positive with antibody anti-Vi, while all Gm(a+) and all Gm(b^2+) [called Gm(f+) by these authors] proteins were positive with antibody anti-We. No myeloma 7S γ-globulin protein was simultaneously positive for more than one of the Gm antigens, a, b^1, or b^2. These observations led Kunkel *et al.* to postulate that $Gm^{b(2)}$ possibly is the allele of Gm^{a} and that Gm(b^1) may be produced by an allele at another locus.

A possible explanation of the association between the Vi antigenic site and Gm(b^1) is that the Gm(b^1) antigen, or antigenic site, cannot be formed in the absence of the Vi antigenic site; similarly Gm(a) and Gm(b^2) might require the presence of the We antigenic site before either can be formed.* There is a suggestion of a similar phenomenon relative to the formation of the Inv protein. It seems that only rarely, if at all, do Type II proteins show Inv activity, while Type I proteins do so frequently. The same pattern seems to hold for the A_1 antigen of the ABO blood groups. This antigen does not form in the absence of the A antigen. Similarly, the f antigen of the Rh blood group system does not form in the absence of the c antigen or the presence of the E antigen (Rosenfield *et al.*, 1962). Many comparable situations exist among the other red blood cell blood group systems (see Race and Sanger, 1962, for examples).

The assumption that two or more loci determine the Gm factors requires several unusual, if not unprecedented, subsidiary hypotheses to explain the family and population data. It also suffers from the disability (not necessarily fatal) of making the Gm polymorphism different in principle from all others. The assumption of a single Gm locus at which a series of alleles determines the several Gm factors and antigens seems to offer the simplest interpretation consistent with all the population and family data. It requires only the assumption that the several antigens and alleles occur with different frequencies in different populations, an assumption in accord with all other information concerning the occurrence of polymorphisms in man and other species. Furthermore, it avoids the contradictions between genetic theory and the observed distribution of the Gm factors in various populations which arise from the multiple-locus hypothesis. Hence, until other evidence is pre-

*See Addendum.

sented, it seems wise to retain the simple assumption that a series of alleles at a single locus determine the Gm factors.

SUMMARY

The Gm(b^2) and Gm(f) types of 255 serum samples from U. S. Negroes and whites; Brazilians of mixed white, Negro and South American Indian ancestry; Bushmen and Bantu from South Africa; and Melanesians from New Guinea were compared. All 255 comparisons were concordant. It is concluded that Gm(f) represents a rediscovery of Gm(b^2).

Consideration of the family and population data for the Gm factors leads to the conclusion that they are produced by alleles at a single locus.

ADDENDUM

Since this manuscript was submitted, Smithies (1965) has suggested that IgG formation may require the transduction of a receptor locus, containing the information for the invariant portion of IgG, by an antibody virus containing information for the variant portion of IgG. If we assume that the We, Vi, etc. specificities of γH chains are in the variant portion of the chain and that Gm(a) [or Gm(b^2)] is, or is intimately related to, the receptor cite for We, and Gm(b^1) for Vi, the data may be explained on the basis of this hypothesis.

REFERENCES

BOYER, S. H., AND YOUNG, W. J. 1961. Gamma globulin (Gm group) heterogeneity in chimpanzees. *Science* 133: 583–584.

GOLD, E. R., MÅRTENSSON, L., ROPARTZ, C., RIVAT, L., AND ROUSSEAU, P.-Y. 1965. Gm(f)—a determinant of human γ-globulin; preliminary communication. *Vox Sang.* 10: 299–302.

HARBOE, M. 1959. A new hemagglutinating substance in the Gm system, anti-Gm^b. *Acta Path. Microb. scand.* 47: 191–198.

KUNKEL, H. G., ALLEN, J. C., CREY, H. M., MÅRTENSSON, L., AND GRUBB, R. 1964. A relationship between the H chain groups of 7S γ-globulin and the Gm system. *Nature* 203: 413–414.

MÅRTENSSON, L. 1964. On the relationships between the γ-globulin genes of the Gm system. A study of Gm gene products in sera, myeloma globulins, and specific antibodies with special reference to Gm(f). *J. Exp. Med.* 120: 1169–1188.

RACE, R. R., AND SANGER, R. 1962. *Blood Groups in Man*, 4th Ed. Philadelphia: F. A. Davis.

ROBBINS, R. B. 1918. Some applications of mathematics to breeding problems. *Genetics* 3: 375–389.

ROPARTZ, C., RIVAT, L., ROUSSEAU, P.-Y., AND LENOIR, J. 1961. Les facteurs Gm a, Gm b, Gm x, "Gm-like" et InV chez les Japonais. *Rev. Franc. Etudes Clin. Biol.* 6: 813–816.

ROPARTZ, C., ROUSSEAU, P.-Y., AND RIVAT, L. 1961. Données actuelles des facteurs sériques Gm dans la monde. *Proc. Second Internat. Cong. Hum. Genet.* 2: 771–772.

ROSENFIELD, R. E., ALLEN, F. H., JR., SWISHER, S. N., AND KOCHWA, S. 1962. A review of Rh serology and presentation of a new terminology. *Transfusion* 2: 287–312.

SMITHIES, O. 1965. Antibody induction and tolerance. *Science*: (in press).

STEINBERG, A. G. 1962. Progress in the study of genetically determined human gamma globulin types (The Gm and Inv groups). *Prog. Med. Genet.* 2: 1–33.

STEINBERG, A. G., AND GOLDBLUM, R. 1965. A genetic study of the antigens associated with the Gm(b) factor of human gamma globulin. *Amer. J. Hum. Genet.* 17: 133–147.

STEINBERG, A. G., AND MATSUMOTO, H. 1964. Studies on the Gm, Inv, Hp and Tf serum factors of Japanese populations and families. *Hum. Biol.* 36: 77–85.

STEINBERG, A. G., AND POLMAR, S. H. 1965. The relation of the S and F fragments, and the H and L chains of gamma globulin to the Gm groups. *Vox Sang.* 10: 369–370.

STEINBERG, A. G., STAUFFER, R., BLUMBERG, B., AND FUDENBERG, H. 1961. Gm phenotypes and genotypes in U. S. whites and Negroes; in American Indians and Eskimos; in Africans; and in Micronesians. *Amer. J. Hum. Genet.* 13: 205–213.

STEINBERG, A. G., AND WILSON, J. 1963. Studies on hereditary gamma globulin factors: Evidence that Gm(b) in whites and Negroes is not the same and that Gm-like is determined by an allele at the Gm locus. *Amer. J. Hum. Genet.* 15: 96–105.