Gm(20), a New Hereditary Gamma Globulin Factor

MARTIN R. KLEMPERER,[1] EUGENIA R. HOLBROOK,[2]
AND H. HUGH FUDENBERG[3]

[1]*The Blood Grouping Laboratory,*
Boston, Massachusetts.
[2]*The Department of Pediatrics, Harvard Medical School,*
Boston, Massachusetts.
[3]*Department of Medicine,*
University of California School of Medicine,
San Francisco, California 94122.

THE DISCOVERY by Grubb (1956) of a heritable human gamma globulin factor has led to the definition of a system of genetically determined antigens present in the human immunoglobulins. This system is presently comprised of two groups of factors determined by codominant alleles at two different loci (Steinberg, 1962; Fudenberg 1963), the Gm factors limited to the IgG species of immunoglobulins, and the Inv factors common to all three species of immunoglobulins (Franklin *et al.*, 1962).

The present paper defines a new human gamma globulin factor Gm(20)* associated with Gm(1) yet distinct from Gm(2) (Harboe and Lundevall, 1959) or Gm(7) (Brantzaeg *et al.*, 1961). The agglutinator, Gar, identifying this antigen was found in the serum of an individual with rheumatoid arthritis.

MATERIALS AND METHODS

The serum samples were (a) sera from 313 unrelated Caucasians, (b) sera from 67 unrelated Negroes, (c) sera from 38 unrelated Chinese, and (d) sera from 53 Caucasian families with a total of 189 offspring.

The gamma globulin typing for Gm(20) was performed using the slide disagglutination technique of Lawler (1960). Typing of other Gm factors was performed using the tube method (Fudenberg, 1963). The reagents used in these systems are listed in Table 1. In the typing of Gm(20), one part packed Group O, Rh_0 cells were incubated with three parts of undiluted anti-D (Nis) of the type Gm(1,−2,3,4,5,20) at 37°C for 15 minutes. The coated cells were then washed three times in saline.

After the last wash, all saline was removed and the cells were kept packed. Enough coated cells were removed to make a 2.5% saline suspension, and this

Received March 7, 1966.

Supported by U. S. Public Health Service research grants GM 09107, HE 05997, and AM 08527 from the National Institutes of Health and by the Life Insurance Medical Research Fund.

*The numerical notation for genetic factors of human immunoglobulins is that recommended by the expert committee of the World Health Organization in: *Bull. Wld. Hlth. Org.* 33: 72–75 (1965); reprinted in *Amer. J. Hum. Genet.* 18: 226–230 (1966).

TABLE 1. REAGENTS USED TO DETERMINE THE GM FACTORS OF γ-GLOBULIN

Factor	Antibody	Dilution	Anti-D	Dilution
Gm(1)	(Mur)	1:4	(Gag)	1:1
Gm(1)	(Mar)	1:4	(Gag)	1:1
Gm(2)	(Gre)	1:4	(Harl)	1:1
Gm(2)	(Pres)	1:4	(Harl)	1:1
Gm(3)	(Cott)	1:2	(DeCo)	1:1
Gm(4)	(Vix)	1:3	(Da)	1:4
Gm(5)	(Har)	1:30	(Han)	1:1
Gm(20)	(Gar)	1:20*	(Nis)	1:1

*This dilution was made using a 1:16 dilution of normal serum with the phenotype Gm(−1,−2,3,4,5,−20) as the diluent.

suspension was used immediately. The rheumatoid serum possessed agglutinator activity against several gamma globulin antigens which have not as yet been identified. Specific agglutinator activity for Gm(20) was achieved by making a 1:20 dilution of the agglutinator in a 1:16 dilution of normal serum with the phenotype Gm(−1,−2,3,4,5,−20). The agglutinating titer against the (Nis) coated cells was unaffected by dilution with this normal serum. One drop of this specific agglutinator was added to one drop of the coated cell suspension, and the mixture was kept at room temperature until strong agglutination was noted. One drop of normal serum (1:4) to be typed was added with thorough mixing. Patterns were read after two to three minutes.

Controls for the detection of an agglutinator in the sera being typed consisted of a drop of 1:4 dilution of serum mixed with one drop of a 2% suspension of coated cells in a 10 × 75 mm test tube. These were incubated at room temperature for 2 hours, spun and read.

RESULTS

Tests of sera from unrelated Caucasian individuals. Samples of serum from 313 unrelated Caucasians of known Gm(1) and Gm(5) types were tested for Gm(20). Of these, 178 (56.9%) were positive for Gm(1). From this group, 147 (82.6%) were positive for Gm(20). All Gm(20) individuals were Gm(1). (See Table 2.) Four individuals homozygous for Gm(1) were found to be Gm(−20). In the Caucasian population tested, the gene frequency of Gm(20) is .2717.

Tests of sera from unrelated Negro individuals. Samples of sera from 67 unrelated Negroes were typed for the gamma globulin factors Gm(1,2,3,5,20) using the reagents listed in Table 1. The gamma globulin factor Gm(20) was present in 66 (98.6%) of these individuals. The phenotypes of those tested are listed in Table 3.

Tests of sera from unrelated Chinese individuals. Samples of serum from 38 unrelated Chinese were typed for the gamma globulin factors Gm(1,2,3,5,20) using the reagents listed in Table 1. The gamma globulin factor Gm(20) was present in all of these individuals. The phenotypes of those tested are listed in Table 4.

Family studies. The sera from 53 Caucasian families with 189 children were

TABLE 2. THE OBSERVED AND EXPECTED NUMBERS OF PHENOTYPES OF UNRELATED CAUCASIAN INDIVIDUALS TESTED FOR GM(20)

	Gm phenotype					
	1,−3,−5,20	1,3,5,20	1,−3,−5,−20	1,3,5,−20	−1,3,5,−20	Total
Observed	39	108	4	27	135	313
Expected	36.9	110.0	2.1	32.9	131.0	312.9

$\chi^2_{(2)} = 3.0551$; $.3 > P > .2$.

TABLE 3. PHENOTYPES OF UNRELATED NEGROES TESTED FOR GM(20)

Number tested	Gm phenotype	Gm(20) (+)	Gm(20) (−)
46	1,−2,−3,−5	45	1
15	1,−2,3,−5	15	0
4	1,2,−3,5	4	0
1	1,−2,−3,−5	1	0
1	1,2,3,5	1	0

TABLE 4. PHENOTYPES OF UNRELATED CHINESE TESTED FOR GM(20)

Number tested	Gm phenotype	Gm(20) (+)	Gm(20) (−)
34	1,−2,3,5	34	0
2	1,2,3,5	2	0
1	1,2,−3,−5	1	0
1	1,−2,−3,−5	1	0

typed for Gm(1), Gm(5), and Gm(20). The data are presented in Table 5. In eight families in which both parents were Gm(−20), all 24 offspring were Gm(−20). Linkage counts were possible in 29 families in which the phenotypes of the parents were Gm(1,5,20) × Gm(−1,5,−20) or Gm(1,5,20) × Gm(1,5,20). In the 100 children born to these parents, Gm(20) segregated with Gm(1). There was no evidence of crossing over.

DISCUSSION

The specificity of the hemagglutination inhibition system used in the phenotyping of human gamma globulin is determined by both the genetically determined antigens of the "incomplete" anti-Rh serum and the antihuman gamma globulin of the agglutinator. The presence of antibodies of differing specificities in a single rheumatoid serum is well known (Ropartz and Hurel, 1959; Harboe, 1960). The rheumatoid arthritis serum containing the agglutinator for Gm(20) also contained agglutinators for other Gm factors. Diluting the agglutinating serum with normal serum of Gm(−1,−2,3,4,5,) phenotype established specificity for the system identifying Gm(20).

The distribution of the Gm(20) antigen in the Caucasian population tested is most closely approximated by the Gm(7) antigen. Of those tested, 82.6% of Gm(1) individuals possessed the Gm(20) antigen while 90% of Gm(1) Cau-

TABLE 5. THE INHERITANCE OF Gm(20) AMONG THE CHILDREN IN 53 CAUCASIAN FAMILIES

Gm genotypes of parents	Number of families	Gm genotypes of offspring					
		1,−5,20	1,5,20	−1,5,−20	1,5,−20	1,−5,20	Total
(1,5,20) × (1,−5,20)	5	9	11				20
(1,5,20) × (1,5,20)	3	2	9	5			16
(−1,5,−20) × (−1,5,−20)	4			12			12
(1,5,20) × (−1,5,−20)	27		49	46			95
(1,5,−20) × (−1,5,−20)	4			10	2		12
(1,−5,20) × (−1,5,−20)	8		24		2		26
(1,5,−20) × (1,−5,20)	2	3	1		2	2	8
	53	14	94	73	6	2	189

casians have been found to have the Gm(7) antigen (Brantzaeg *et al.*, 1961). Among Negroes, Gm(7) is a common antigen (Steinberg *et al.*, 1962; Fudenberg, 1963). In this study, Gm(20) was found in 98.6% of Negroes tested. However, it is unlikely that Gm(20) and Gm(7) are identical antigens. The serum of a known Gm(1,−7) Caucasian individual consistently typed positive for Gm(20). We have found two Gm(1,−2,−3,−5) and two Gm(1,2,−3,−5) individuals who were Gm(−20). Deicher *et al.* (1963) observed no individuals of the phenotype Gm(1,−2,−5,−7) or Gm(1,2,−5,−7) in a study of the inheritance of Gm(1), Gm(5), Gm(2), and Gm(7). Thus, Gm(20) appears to be an antigen distinct from Gm(7). It also appears to be distinct from Gm(9) in that all four possible types, i.e. Gm(9,20), Gm (−9,−20), Gm(9,−20), and Gm(−9,20) have been found in testing a standard panel of donor sera (M. Waller, personal communication).

The distribution of Gm(20) in the Caucasian population can be described by the three alleles Gm^1, $Gm^{1,20}$ and Gm^5. The frequencies of these alleles in this population can be estimated by use of the maximum likelihood equations (Stevens, 1938). If the frequency of

$$Gm^1 = p$$
$$Gm^{1,20} = q$$
$$Gm^5 = r$$

then

$$p = 1 - q - r$$

$$q = 1 - \sqrt{1 - \overline{Gm}(1,-5,20) - \overline{Gm}(1,5,20)}$$

$$r = \tfrac{1}{2}\,\overline{Gm}(1,5,-20) + \tfrac{1}{2}\,\overline{Gm}(1,5,20) + \overline{Gm}(-1,5,-20).$$

where $\overline{Gm}$ refers to the frequency of the specified phenotype. The estimates derived from the data in Table 2 are: $p = .0813$, $q = .2717$, and $r = .6470$. These gene frequencies can then be used to determine the expected numbers for each of the five possible phenotypes.

The family data are consistent with the hypothesis that the presence of Gm(20) is inherited as a dominant trait. Thus all 24 children of Gm(−20) × Gm(−20) matings were Gm(−20); the children of Gm(20) × Gm(20) mat-

ings could be either Gm(20) or Gm(−20), since some of the Gm(20) parents are heterozygous.

Studies with isolated normal IgG, IgA, and IgM globulins and with 12 γG, 12 γA, and 12 γM paraproteins demonstrated the presence of the Gm(20) antigen to be present only in IgG globulin and γG paraproteins. In these proteins, the Gm(20) antigen was localized in the Fc fragment (Fudenberg and MacKenzie, unpublished data).

SUMMARY

A new heritable human gamma globulin factor is described. It is designated Gm(20) and, like Gm(2) and Gm(7), is present only in Gm(1) individuals. This factor was studied in 313 unrelated Caucasians, 67 unrelated Negroes, 38 unrelated Chinese, and 53 Caucasian families with a total of 189 offspring. Some 82.6% of Gm(1) Caucasians, 98.5% of Negroes and 100% of Chinese were found to be Gm(20)-positive. In family studies, the factor was transmitted as a dominant trait closely linked to Gm(1).

ACKNOWLEDGMENT

The authors gratefully thank Helen Moulton and Patricia A. Corcoran for their invaluable assistance and advice.

REFERENCES

BRANTZAEG, B., FUDENBERG, H., AND MOHR, J. 1961. The Gm(r) serum group. *Acta Genet. Stat. Med.* (Basel) 11: 170–177.

DEICHER, H., WENDT, G. G., THEILE, U., AND KIRCHBERG, G. 1963. Familienuntersuchungen über die Gammaglobulingruppen Gm(a), Gm(b), Gm(x) and Gm(r). *Acta Genet. Stat. Med.* (Basel) 13: 124–131.

FRANKLIN, E. C., FUDENBERG, H., MELTZER, M., AND STANWORTH, D. R. 1962. The structural basis for genetic variations of normal human gamma globulins. *Proc. Nat. Acad. Sci.* (U. S.) 48: 914–922.

FUDENBERG, H. 1963. The hereditary human gamma globulin (Gm) groups: Interpretations and extensions. *Prog. Allergy* 7: 1–31.

GRUBB, R. 1956. Agglutination of erythrocytes coated with "incomplete" anti-Rh by certain rheumatoid arthritic sera and some other sera. The existence of human serum groups. *Acta Path. Microb. Scand.* 39: 195–197.

HARBOE, M. 1960. Simultaneous occurrence of hemagglutinating substances of different specificities in rheumatoid sera. *Acta Path. Microb. Scand.* 49: 381–392.

HARBOE, M., AND LUNDEVALL, J. 1959. A new type in the Gm system. *Acta Path. Microb. Scand.* 47: 357–370.

LAWLER, S. D. 1960. A genetical study of the Gm groups in human serum. *Immunology* 3: 90–94.

ROPARTZ, C., AND HUREL, R. 1959. Influence de l'anti-Rh et du sérum de polyarthrite chronique evolutive dans le choix du complexe agglutinant destiné a l'exploration du facteur sérique Gm. *Rev. Franc. Études Clin. Biol.* 4: 280–282.

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., WILSON, J., AND LANSET, S. 1962. A new human gamma globulin factor determined by an allele at the Inv locus. *Vox Sang.* 7: 151–156.

STEVENS, W. L. 1938. Estimation of blood group gene frequencies. *Ann. Eugen.* (Lond.) 8: 362–375.