whereas the S fragment contains the L-chain and part of the H-chain (*7*). The specific Cr-determinants and Zu-determinants were found on the F (fast) fragment. Since the Cr- and Zu-proteins differ markedly in carbohydrate content, further study may implicate this moiety in the antigenic differences.

Serums of 150 normal individuals were examined, and all contained both populations of γ-globulin. Several of these serums were known to be of different Gm specificities. The results indicated that both antigenic types of 7*S* γ-globulins (Cr and Zu) are present, regardless of the Gm type of the serum.

These findings are consistent with the assumption that in normal human serums at least two populations of 7*S* γ-globulins are present which differ in part of the antigenic structure of their H-chains. To date it is impossible to state whether any more different types of H-chains of 7*S* γ-globulins exist, as appropriate antiserums to study this problem have not been available. However, an antiserum prepared against the reduced and alkylated 7*S* γ-globulin of one individual gave two lines in immunodiffusion experiments with 7*S* γ-globulin, which fused with the two lines of the reaction of antiserum to Zu with normal human serum.

The two antigenically distinguishable types of H-chains in normal and myeloma 7*S* γ-globulins may be compared to the two types of L-chains in these molecules. However, neither Cr-determinants nor Zu-determinants are confined to one or the other of the antigenic type I or type II classes.

It is proposed that these two populations of normal and myeloma 7*S* γ-globulins be designated 7*S* γ_{Cr} and 7*S* γ_{Zu}, at least until a functional name is applicable.

RUDY E. BALLIEUX
GEORGE M. BERNIER
KIKUO TOMINAGA
FRANK W. PUTNAM
Department of Biochemistry, University of Florida College of Medicine, Gainesville

References and Notes

1. S. Migita and F. W. Putnam, *J. Exptl. Med.* **117**, 81 (1963); M. Mannik and H. G. Kunkel, *ibid.* **117**, 213 (1963); J. L. Fahey, *J. Immunol.* **91**, 448 (1963); F. W. Putnam, C. W. Easley, J. W. Helling, *Biochim. Biophys. Acta* **78**, 231 (1963).
2. H-chain is used to signify that part of the 7*S* γ-globulin molecule which is not L-chain.
3. E. C. Franklin, H. H. Fudenberg, M. Meltzer, D. Stanworth, *Proc. Natl. Acad. Sci. U.S.* **48**, 914 (1962); M. Harboe, C. K. Osterland, H. G. Kunkel, *Science* **136**, 979 (1962); S. Cohen, *Nature* **197**, 253 (1963).
4. E. C. Franklin, M. Meltzer, F. Guggenheim, J. Lowenstein, *Federation Proc.* **22**, 264 (1963).
5. E. F. Osserman and K. Takatsuki, *Medicine* **42**, 357 (1963); E. C. Franklin, J. Lowenstein, B. Bigelow, M. Meltzer, *Am. J. Med.*, in press.
6. E. F. Osserman and K. Takatsuki, *ibid.*, in press.
7. G. M. Edelman and B. Benacerraf, *Proc. Natl. Acad. Sci. U.S.* **48**, 1035 (1962).
8. We thank Dr. E. C. Franklin for the abnormal urinary protein of patient Cr, and Dr. E. F. Osserman for specimens of the urinary protein of patient Zu and of 25 7*S* γ myeloma serums. We also thank both for making manuscripts available to us before publication. Drs. A. Steinburg and W. V. Epstein provided human serums of known Gm type. Supported in part by grants H-02966 and CA-02803 of NIH. One of us (R.E.B.) acknowledges a grant from the Netherlands Organisation for the Advancement of Pure Research (Z.W.O.).

26 March 1964

Antibody to Hereditary Human Gamma-Globulin (Gm) Factor Resulting from Maternal-Fetal Incompatibility

Abstract. *Multiple samples of serum from a Gm(a−) female mated to a Gm(a+) male were obtained before, during, and after each of four normal uncomplicated pregnancies and tested for antibody to human gamma globulins of differing genetic types. An agglutinator for the Gm(a) factor first appeared in the mother's serum during the third trimester of the fourth pregnancy. The newborn (male) was genotypically Gm(a+), since his serum contained, in addition to maternal Gm(a−) gamma globulin, small amounts of Gm(a+) gamma globulin.*

Antigenic differences in human 7*S* γ-globulin, detectable by inhibition of agglutination methods, are determined by codominant alleles at two independent loci (Gm and Inv) (*1*). Antibodies to γ-globulin, specific for one or another of these hereditary antigens, have been found in the serums of multiply transfused individuals (*2*), in some Gm(−) offspring of Gm(+) mothers (*3*), in children given multiple injections of pooled Cohn fraction II for "allergy" (*4*), and in children studied 1 to 2 years after exchange transfusion for hemolytic disease of the newborn (*4*). Most reports state that the 7*S* γ-globulin in the human fetus is acquired from the mother by transplacental passage, so that the γ-globulin phenotype (Gm type) of mother and newborn child are identical (*1, 5*); however, endogenous production of small amounts of γ-globulin (0.1 to 1.0 percent of the total present in cord blood) by the fetus has recently been demonstrated in this laboratory (*6*) with the use of selected SNagg (agglutinating serums from nonarthritic patients) reagents (*7*). In such systems Gm(+) normal serums still show inhibitory activity when diluted up to 500- to 2000-fold with saline or Gm (−) serum, and Gm(+) cord serums inhibit at 1:2 to 1:20 dilutions.

These findings suggested that "anti–γ-globulins" might result from immunization of the mother by fetal γ-globulin bearing hereditary Gm determinants elaborated by genes inherited from the father but not present in the mother. In our study, the Gm types of the parents (the authors) (Table 1) made it likely that some of their offspring would be positive for the Gm(a) factor present in the father (HHF) but absent in the mother (BRF). For this reason, multiple samples of serum were obtained from the mother when she was nulliparous and also during and after each of her four pregnancies and stored in portions in the frozen state. After each pregnancy a portion of each of the available samples was thawed and tested simultaneously for anti-Gm activity. Such activity did not appear in the mother's serum during or after the first three pregnancies, but these offspring are Gm(a−)—confirmed by Gm typing at 3 years of age—(Table 1). The fourth pregnancy was uncomplicated and the mother received no transfusions or injections during its course. Her serum did not contain rheumatoid factor (negative results in sensitized sheep-cell and latex-fixation tests). During the fourth pregnancy, however, an antibody to γ-globulin demonstrable by agglutinating systems was detected in the maternal serum at the beginning of the third trimester (Table 2) and persisted until the 35th week of gestation. This agglutinator was specific for the Gm(a) factor; it agglutinated Rh-positive red cells sensitized by Gm(a+) "incomplete" anti-Rh reagents but did not agglutinate cells coated with Gm(a−) anti-Rh. That the agglutinator was specific for Gm(a) was documented by the inhibition obtained with a panel of 25 standard serums of known Gm and Inv types; these serums were used to inhibit the agglutination system established with the mother's serum and Rh-positive cells sensitized by a Gm(a+)

anti-Rh coat (SW). All Gm(a+) serums but no Gm(a−) serums inhibited this agglutination system. Presumably production of agglutinator to Gm(a) was stimulated by the presence of fetal blood in the maternal circulation; evidence for maternal immunization by components of fetal blood is provided by the increase in titer of agglutinins for red cells of blood group B during the fourth pregnancy (mother, blood group A; infant, AB). As shown in Table 2, serum samples obtained at term and at weekly intervals thereafter lacked significant amounts of anti-Gm(a) and showed progressively lower anti-B titers. The cord serum was weakly inhibitory in the Gm(a) test system (Table 3).

These data demonstrate that endogenous synthesis of γ-globulin by the fetus in amounts sufficient to immunize a mother of "incompatible" Gm type is already occurring by the 7th month of gestation. Accordingly, the production and incidence of anti-Gm agglutinators will depend on maternal and fetal Gm genotype, differential "antigenicity" of the various γ-globulin factors, and individual differences (perhaps genetically determined) in antibody response. That there may be a familial predisposition to the formation of agglutinator (or other "anti-" component) to γ-globulin has already been suggested (*8*), and this predisposition may pertain here. Of the three older children, all male and all Gm(a−) (Table 1), the youngest, age 3, developed agglutinator to Gm(a) after injection of 16 percent Cohn fraction II, in a dose of 0.02 ml per kilogram of body weight, whereas the other two boys, after similar injection of Cohn fraction II in doses of 0.02 and 0.04 ml per kilogram at comparable ages, did not develop agglutinators for γ-globulins.

This is, to our knowledge, the first reported instance of the documented appearance of an agglutinator after antigenic stimulation by Gm-incompatible γ-globulin in an individual whose serum, by test, contained no agglutinator previously. The incidence of such a phenomenon is unknown, but probably is low. The maximum titer (1:8) of the mother's serum at 9 months' gestation and the barely detectable reaction with undiluted serum at term may be pertinent in this connection. It is probable that the presence of anti-Gm agglutinins with comparable activity would not have been detected in previous studies of Gm types of paired mother-infant serums (*1*, *5*) in which tests were performed only on postpartum serums and only at dilutions of 1:4 or greater.

The results of our study provide further documentation on the antigenicity of Gm(+) γ-globulin in presumably normal Gm(−) individuals. Although Steinberg and Wilson's data suggest that, in retrospect, an anti-Gm agglutinin was present in one normal subject for at least 30 years (*3*), there is a paucity of data on the long-term consequences of anti-Gm antibodies in the serum of individuals with a predisposition to connective tissue disorders (*8*) and other diseases. Furthermore, agglutinators directed against γ-globulin of one or another genetic type are occasionally present in the 7*S* (γ_2-) component of the immune globulins (*6*).

Since 7*S* γ-globulin passes the placenta, maternal production of 7*S* agglutinins directed against genetic (Gm) determinants in fetal γ-globulin may be important in the etiology of the "functional" (transient) hypogammaglobulinemia of infancy. Such anti-Gm agglutinators have been detected in the 7*S* γ-globulins of two of the mothers of infants with "functional" hypogammaglobulinemia studied in our laboratory (*9*).

Table 1. Blood and serum groups of family F.

Member	Age	Blood group		Type of gamma globulin	
		ABO	Rh	Gm	Inv
Father	35	BO	+	(a+b+x−)	(a−b+)
Mother	37	AO	+	(a−b+x−)	(a+b+)
Son	7	O	−	(a−b+x−)	(a−b+)
Son	5	B	+	(a−b+x−)	(a+b+)
Son	3	B	+	(a−b+x−)	(a+b+)
Son	*	AB	+	(a+b+x−)	?

* Newborn.

Table 2. Isoagglutinin (anti-B) and anti-Gm(a) titers of mother's serum before and after birth of fourth son.

Time	Anti-B	Anti-Gm(a)
Pregnancy		
−2 mo	32	0
+2 mo	32	0
+5 mo	32	0
+7 mo	128	4
+9 mo	256	8
+9½ mo	256	4
Term	256	½*
Postpartum		
1 wk	2048	½*
2 wk	1024	0
3 wk	640	0
4 wk	640	0

* Plus-minus reaction with undiluted serum.

Table 3. Inhibition of Gm(a) agglutination system by cord serum diluted from 1:1 to 1:2000 in Gm(a−) serum. The agglutination system contained anti-Gm(a) No. 51 and anti-Rh SW. The system was controlled as follows: anti-Gm(a) and sensitized cells resulted in no inhibition; saline and sensitized cells, anti-Gm(a) and uncoated cells, inhibitor and sensitized cells each resulted in no inhibition. Agglutination was graded from 0 to ++; ++ = no inhibition, + = partial inhibition, 0 = inhibition. Similar results were obtained with anti-Gm(a) Con.

1	2	4	8	16	32	64	125	250	500	1000	2000
Cord serum											
0	0	0	+	++	++	++	++	++	++	++	++
Newborn's serum at 3 months											
0	0	0	0	0	0	0	+	++	++	++	++
Gm(a+b+) positive control											
0	0	0	0	0	0	0	0	0	0	+	++
Gm(a−b+) negative control											
++	++	++	++	++	++	++	++	++	++	++	++

H. H. Fudenberg
Betty Roof Fudenberg
Department of Medicine, University of California School of Medicine, San Francisco 22

References and Notes

1. A. G. Steinberg, *Progr. Med. Genetics*, **2**, 1 (1962); H. H. Fudenberg, *Progr. Allergy* **7**, 1 (1963).
2. J. C. Allen and H. G. Kunkel, *Science* **139**, 418 (1963); H. H. Fudenberg and H. Perkins, unpublished.
3. A. G. Steinberg and J. A. Wilson, *Science* **140**, 303 (1963).
4. E. R. Stiehm and H. H. Fudenberg, in preparation.
5. P. Linnet-Jepsen, F. Galatius-Jensen, M. Hauge, *Acta Genet. Statist. Med.* **8**, 164 (1958); M. Harboe and J. Lundevall, *Acta Pathol. Microbiol. Scand.* **45**, 357 (1959); J. Moullec, R. Kherumian, E. Sutton, P. Espagnon, *Rev. Hematol.* **11**, 512 (1956).
6. L. Mårtensson, Program, Annual Meeting of the American Society of Human Genetics, New York, 19–21 July 1963, p. 6.
7. C. Ropartz, J. Lenoir, Y. Hemet, L. Rivat, *Nature* **188**, 1120 (1960).
8. H. H. Fudenberg and E. C. Franklin, *Ann. N.Y. Acad. Sci.*, in press.
9. H. H. Fudenberg and R. A. Good, unpublished.
10. Supported by ONR contract Nonr-3656(12) (NR 105-308), USPHS grant H-5997, the California division of the American Cancer Society, and the University of California Statewide Cancer Coordinating Committee.

9 April 1964