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# Antibodies against γ-Globulin after Repeated Blood Transfusions in Man *

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Agglutinators specific for genetically controlled antigenic determinants of human γ-globulin are found in serum from patients with rheumatoid arthritis and less frequently in serum from clinically normal donors (1–3). Such agglutinators have been used to define a number of genetic determinants that occur as the Inv factors on the L polypeptide chain and the Gm factors on the H polypeptide chain of human γ-globulin (4–6).

Recently a high incidence of Gm-specific agglutinators has been found in children who have received repeated blood transfusions (7, 8). This suggested that genetically foreign isologous γ-globulin was antigenic in man. Subsequent studies have indicated that placentally transferred, genetically foreign γ-globulin from the mother can immunize the human fetus (9–11). Antigenicity of isologous γ-globulin in a variety of other animal species has been well established since studies by L'Oudin delineated the genetically controlled allotypes of rabbit γ-globulin (12). More recently, a number of workers have shown that autologous γ-globulin may be made antigenic in the rabbit by a variety of physical or chemical alterations of the γ-globulin molecules (13–15). The development of anti-γ-globulin antibodies after immunization of animals (13, 16) and man (17) with a variety of nonglobulin antigens has been demonstrated, presumably due to antigenicity of antigen-antibody complexes.

The frequent occurrence of anti-Gm agglutinators in serum from patients with rheumatoid arthritis (18) has suggested a close relationship between Gm-specific and other anti-γ-globulin activity in these sera. The studies that form the basis of this report were designed to explore the occurrence of various types of anti-γ-globulin activity in repeatedly transfused humans in the hope of further elucidating their origin, nature, and relationship. Results have shown that not only do children repeatedly exposed to isologous γ-globulin have a high incidence of specific anti-Gm serum agglutinating activity, but that, in addition, rheumatoid factor-like antibodies of undetermined specificity appear in a large percentage of children and adults so treated.

## Methods

Serum samples were obtained from children and adults who had received multiple blood transfusions as whole blood or packed erythrocytes. Age of the patient, nature of the transfused blood, frequency and duration of transfusions, and information as to historical or physical evidence of joint disease were ascertained whenever possible. Serum samples were obtained from mothers of a number of the children who had donated blood for study. All serum samples were stored at −20° C and made 1:10,000 with sodium azide to impede bacterial contamination.

Hemagglutination procedures were performed in a standard manner as previously reported from this laboratory (19). In brief, the basis of Gm typing of human γ-globulin lies in the ability of sera with Gm-specific antibody to agglutinate human group O, Rh-positive erythrocytes that have been coated with an incomplete human anti-Rh (anti-D) antibody carrying the Gm factor to be detected. Thus, a serum with anti-Gm(a) activity will agglutinate erythrocytes coated with an incomplete anti-Rh antibody whose γ-globulin molecules themselves are Gm(a) positive. Unbound Gm(a)-positive γG-globulin molecules present in the reaction mixture will bind preferentially with the Gm(a)-specific agglutinating antibody and prevent agglutination of the coated erythrocytes. Thus, the Gm phenotype of a serum is assessed by its ability to inhibit standard hemagglutinating systems specific for the various Gm factors. The standard reagent systems used for typing the genetic factors used in this study, and the dilutions at which they were utilized, are shown in Table I. In a similar manner, the specificity of an agglutinating serum can be established by studying the ability of a bank of normal, nonagglutinating sera of known Gm phenotype to inhibit its ability to agglutinate appropriately coated erythrocytes.

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TABLE I

*Reagent systems used to type genetic factors*

| Factor to be typed | Agglutinating antibody in serum | Anti-D coating antibody in serum |
|---|---|---|
| Gm(a) | Smejsa (1:30 dilution) | 2368 (1:5 dilution) |
| Gm(b) | A. Berg (1:20 dilution) or Bomb. (1:80 dilution) | Black (1:5 dilution) |
| Gm(x) | Shio. (1:25 dilution) | 2368 (1:5 dilution) |

Strong anti-D antibodies (titer 1:160 or greater) of broad and limited reactivity for rheumatoid factor, as well as those of known Gm specificity, were used to screen the various sera for hemagglutinating antibodies. Group O, $Rh_o$ erythrocytes were coated with these anti-D coats by incubating washed cells for 2 hours at 37° C with 1:5 dilutions of the anti-Rh sera. Sera to be checked for hemagglutinating activity were reacted with various anti-D coated erythrocytes when undiluted, and diluted 1:5 and 1:10, by a slide technique. Hemagglutinating titers were established in serial twofold dilutions. Specificity of Gm-specific agglutinating activity for those Gm factors studied here was established by inhibition studies utilizing a panel of 20 normal sera of known Gm and Inv phenotypes. In all instances sera were checked for agglutination of uncoated O, $Rh_o$ cells to exclude anti-red cell antibody, and no such activity was ever demonstrated.

Gm phenotypes of γ-globulin in sera were established by inhibition studies utilizing the Gm reagents of known specificity listed above. When agglutinating activity in the serum to be typed interfered with the typing procedure, one or more of three procedures was used. Sera were diluted beyond the titer of their agglutinating activity, when this dilution fell within the sensitivity of the typing system, or an aliquot of serum was dialyzed against 0.1 M 2-mercaptoethanol overnight, followed by

TABLE II

*Anti-γ-globulin activity in sera from repeatedly transfused children*

| Gm phenotype | Diagnosis | Age | Maternal Gm phenotype (if known) | Approximate no. transfusions | Anti-γ-globulin activity | | |
|---|---|---|---|---|---|---|---|
| | | | | | Anti-Gm specificity | Anti-Gm titer | Latex fixation titer |
| | | *years* | | | | | |
| (a−b+x−) | Thalassemia major | 5 | | 30 | a | 1:2,560 | 1:320 |
| | | 14 | | >200 | a | 1:1,280 | 1:80 |
| | | 8 | a−b+x− | >100 | a | 1:320 | 1:640 |
| | | 14 | a+b+x− | >100 | a | 1:320 | 1:640 |
| | | 11 | a−b+x− | 90 | a | 1:320 | 1:320 |
| | | 3 | | >10 | a | 1:320 | 1:320 |
| | | 7 | | 50 | a | 1:320 | 1:40 |
| | | 3 | a−b+x− | 10 | a | 1:320 | 0 |
| | | 10 | | 50 | a | 1:320 | 0 |
| | | 9 | a+b+x+ | >100 | a | 1:160 | 1:640 |
| | | 13 | a+b+x+ | >100 | a | 1:160 | 1:320 |
| | | 14 | a−b+x− | >200 | a | 1:160 | 1:40 |
| | | 2 | a−b+x− | >20 | a | 1:160 | 1:40 |
| | | 20 | a−b+x− | >300 | a | 1:160 | 0 |
| | | 13 | | >100 | a | 1:160 | 0 |
| | | 17 | | >200 | a | 1:80 | 1:160 |
| | | 14 | | >100 | a | 1:80 | 1:80 |
| | | 18 | a−b+x− | >300 | a | 1:80 | 0 |
| | | 3 | | >5 | a | 1:80 | 0 |
| | | 8 | a−b+x− | 50 | a | 1:40 | 0 |
| | | 5 | a−b+x− | 70 | a | 1:40 | 0 |
| | | 6 | a−b+x− | 70 | a | 1:40 | 0 |
| | | 3 | a+b+x− | 10 | a | 1:20 | 0 |
| | | 10 | | Multiple, no. unknown | a | 1:20 | 0 |
| | | 8 | a−b+x− | 70 | a | 1:10 | 0 |
| | | 6 | | 40 | a | 1:10 | 0 |
| | | 8 | | Multiple, no. unknown | a | 1:10 | 0 |
| | | 22 | | >100 | a | 1:10 | 0 |
| | | 13 | a−b+x− | >200 | None | 0 | 0 |
| | | 10 | a+b+x− | 70 | None | 0 | 0 |
| | | Unknown | | Multiple, no. unknown | None | 0 | 0 |
| | | 11 | | Multiple, no. unknown | None | 0 | 0 |
| | Congenital hemolytic anemia | 6 | | >20 | a | 1:20 | 0 |
| | | 4 | | >10 | a | 1:20 | 0 |
| | Acute leukemia | 8 | | >5 | x | 1:5 | 0 |

TABLE II—(*Continued*)

| Gm phenotype | Diagnosis | Age | Maternal Gm phenotype (if known) | Approximate no. transfusions | Anti-γ-globulin activity | | |
|---|---|---|---|---|---|---|---|
| | | | | | Anti-Gm specificity | Anti-Gm titer | Latex fixation titer |
| | | *years* | | | | | |
| (a+b+x−) | Thalassemia major | 17 | | >100 | x | 1:320 | 1:640 |
| | | 8 | a+b+x− | 90 | x | 1:80 | 1:160 |
| | | 9 | | 80 | x | 1:40 | 0 |
| | | 19 | a+b+x− | >100 | x | 1:10 | 0 |
| | | 6 | a+b+x− | <20 | x | 1:10 | 0 |
| | | 3 | | Multiple, no. unknown | x | 1:10 | 0 |
| | | 2 | a−b+x− | Multiple, no. unknown | x | 1:5 | 0 |
| | | 21 | | >200 | None | 0 | 1:80 |
| | | Unknown | | Multiple, no. unknown | None | 0 | 1:80 |
| | | 1 | a+b+x− | 3 | None | 0 | 0 |
| | | 6 | a−b+x− | >10 | None | 0 | 0 |
| | | 8 | a−b+x− | 20 | None | 0 | 0 |
| | | 5 | | 30 | None | 0 | 0 |
| | | 8 | | Multiple, no. unknown | None | 0 | 0 |
| | | 9 | | >100 | None | 0 | 0 |
| | | 1 | | Multiple, no. unknown | None | 0 | 0 |
| | | 15 | | >100 | None | 0 | 0 |
| | | 3 | | 10 | None | 0 | 0 |
| | Congenital hemolytic anemia | 8 | | Multiple, no. unknown | None | 0 | 0 |
| | Acute leukemia | 2 | | 4 | None | 0 | 0 |
| | Sickle cell anemia | 6 | a+b+x− | 2 | None | 0 | 0 |
| | | 16 | | >10 | None | 0 | 0 |
| | | 7 | a+b+x− | 3 | None | 0 | 0 |
| | | 7 | a+b+x− | 2 | None | 0 | 0 |
| | | 7 | | Multiple, no. unknown | None | 0 | 0 |
| (a+b−x−) | Thalassemia major | 13 | | >200 | b | 1:10 | 1:80 |
| | | 9 | | 36 | None | 0 | 0 |
| | | Unknown | | Multiple, no. unknown | None | 0 | 0 |
| (a+b+x+) | Thalassemia major | 12 | | >100 | None | 0 | 0 |
| | | 7 | | 50 | None | 0 | 0 |

dialysis for 8 hours against 0.02 M iodoacetamide and then by dialysis against normal saline. Volume changes were measured carefully and allowances made in subsequent dilutions for inhibition studies. In all instances in which it was used, this treatment removed or lowered the titer of agglutinating activity to allow performance of genetic phenotyping. Such treatment has been shown to dissociate γM-globulins and γM-antibodies to small and inactive or less active units (20, 21). In certain instances, 7 S peaks from sucrose gradient ultracentrifugation of whole serum, devoid of agglutinating activity, were adjusted to a protein concentration of 1 mg per ml and typed by the inhibition techniques described.

Isolation of γ-globulin was performed by zone electrophoresis in starch (22). Aggregation of γ-globulin solutions at a concentration of 10 mg per ml was accomplished by immersing the solutions in a water bath at 62° to 64° C for 11 minutes. The latex fixation test utilized human Cohn Fraction II after the method of Singer and Plotz (23) starting at a serum dilution of 1:20. All protein concentrations were determined by the Folin-Ciocalteu method (24). Double diffusion in agar was performed as previously described (25). Sucrose density gradient separations were performed as before (26) and harvested from the bottom of the tube. In certain instances to exclude the possibility of contamination during harvesting, the density gradient tube was illuminated from below and fractions were obtained through a capillary pipette introduced to the desired region from the top.

## Results

*Studies in children.* Results of studies on sera from 65 children who had received multiple transfusions are presented in Table II. Fifty-five of these children had thalassemia major, five had sickle cell anemia, three had congenital hemolytic anemia, and two had acute leukemia. Detailed in-

formation as to the nature of blood transfused into these children is not available. Physicians supplying most of the samples indicated that packed cells were frequently used due to the incidence of transfusion reactions, although all cases had received whole blood at some time or another in their course. Thirty-nine of the 65 children were found to have agglutinating activity specific for either Gm(a), Gm(x), or Gm(b).

Of 35 children whose own γG-globulin was of phenotype Gm(a − x −), 30 had sera with anti-Gm(a) activity ranging in titer from 1:10 to 1:2,560. Seven of the 25 children whose phenotypes were Gm(a + x −) and one of the 35 of phenotype Gm(a − x −) had anti-Gm(x) agglutinating activity. It was not possible to exclude the presence of anti-Gm(a) as well as anti-Gm(x) agglutinators in this group, as no serum of phenotype Gm(a − x +) was available for inhibition studies. However, no decrease in agglutinating titer could be detected after inhibition of a number of these agglutinators with Gm(a + x −) sera, and it is felt unlikely that agglutinators against both (a) and (x) were present. One of three patients of phenotype Gm(b −) was found to have a weak anti-Gm(b) agglutinator. No agglutinators for Gm(a), (b), or (x) were found in sera of phenotoype Gm(a + b + x +). Careful search for anti-Inv(a) agglutinators in a number of these patients was unrewarding.

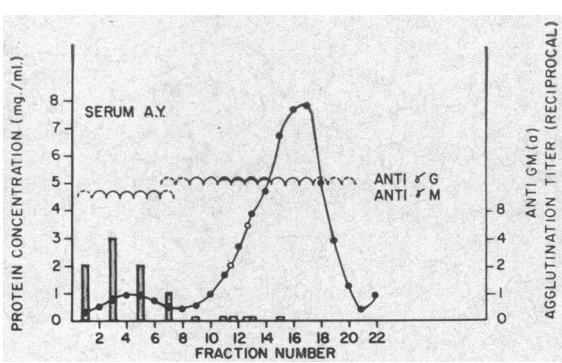


FIG. 1. SUCROSE DENSITY GRADIENT SEPARATION OF SERUM FROM REPEATEDLY TRANSFUSED PATIENT A.Y. WITH THALASSEMIA MAJOR. No detectable anti-Gm(a) agglutinating activity is present except in the area containing γM-globulin identified by diffusion in agar against specific antiserum (as indicated by arcs in upper part of Figure). Open bars and dots represent samples aspirated from top of tube. Anti-Gm(a) activity of this serum was completely removed by treatment with 0.1 M 2-mercaptoethanol.

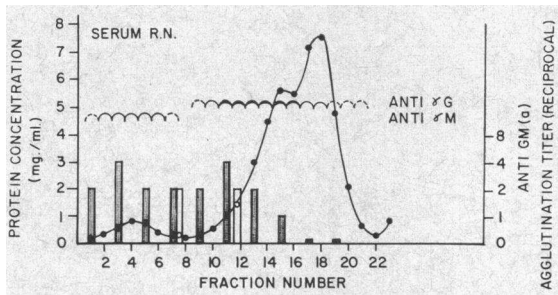


FIG. 2. SUCROSE DENSITY GRADIENT SEPARATION OF SERUM FROM THALASSEMIA PATIENT R.N. Anti-Gm(a) activity is present in regions above those occupied by γM-globulin. Anti-Gm(a) activity at low titer remained after treatment of whole serum with 2-mercaptoethanol.

As a control, sera were also collected from 52 children between the ages of 9 months and 14 years who had been hospitalized for a variety of reasons, none of whom gave a history of parenteral γ-globulin administration or transfusion. Twenty of these children had γG-globulin of phenotype Gm(a − x −), of whom one had anti-Gm(a) activity to a titer of 1:40. This 15-month-old child with an undiagnosed form of ataxia had received no transfusions; his mother was of phenotype Gm(a + x −). One other child in this group had a weak agglutinator detectable only in the undiluted serum, which was possibly specific for the Gm(a) factor, but this could not be determined with certainty because of the low agglutination titer. No agglutinating activity was detectable with the anti-Rh coats used among 24 sera of phenotype Gm(a + b + x −), four sera of phenotype Gm(a + b − x −), and four sera of phenotype Gm(a + b + x +) in this group.

*Role of maternal γ-globulin.* To explore the possible role of placentally transferred maternal γ-globulin, blood samples were obtained from mothers of 27 of the repeatedly transfused children in the study, as shown in Table II. Eleven of 15 children in this group whose serum had anti-Gm(a) activity had mothers whose phenotype was Gm(a −), and one child who was himself Gm(a −) but who failed to develop anti-Gm activity after repeated transfusions had a mother who was Gm(a +). Of the patients whose maternal phenotypes were known, four with anti-Gm(x) agglutinating activity had mothers of phenotype Gm(x −).

*Character of specific agglutinating antibodies.* Studies were performed to determine the class of

agglutinating antibodies found in these sera. Twenty-four samples from repeatedly transfused children with anti-Gm(a) activity were reduced with 2-mercaptoethanol and blocked with iodoacetamide as described above. Such treatment has been shown to dissociate γM-globulin molecules to inactive or less active units (20, 21). Twenty-two of these samples had no demonstrable agglutinating activity remaining after reduction and blocking. Two sera retained weak but definite agglutinating activity specific for the Gm(a) factor. To explore the implications of this observation, sucrose density gradient separations were performed on untreated aliquots from six sera, including these two. The location of γM- and γG-globulins was marked by double diffusion in gel against antisera specific for these immunoglobulin classes. Representative results are presented in Figures 1 and 2. In serum from patient A.Y. (Figure 1) no anti-Gm(a) activity remained after mercaptoethanol treatment, and all detectable agglutinating activity in unreduced serum was restricted to the area of the immunologically identifiable γM-globulins. Identical results were obtained with each of three other anti-Gm(a) sera in which agglutinating activity was totally susceptible to reduction by 0.1 M mercaptoethanol. Anti-Gm(a) activity in the serum from patient R.N. (Figure 2) was reduced from a titer of 1:320 to 1:5 by mercaptoethanol treatment. Density gradient separation of untreated serum from this patient revealed definite anti-Gm(a) activity outside the region of detectable γM-globulin and within fractions of slower sedimentation rate. Similar results were obtained in serum from patient J.M., in which anti-Gm(a) activity titered at 1:1,280 was reduced to 1:5 by 2-mercaptoethanol treatment and in which definite agglutinating activity was present outside the density gradient area of the γM-globulins. Aspiration of density gradient fractions from the tube top gave identical results, as shown in Figures 1 and 2.

Agglutination titers found in fractions above the γM-globulin region of the sucrose gradient on serum from R.N. would suggest that a larger fraction of agglutinating activity resides in the lighter immunoglobulin classes than is apparent from residual anti-Gm(a) titer after mercaptoethanol treatment. The reason for this discrepancy is not known, but may be due to nonspecific loss of titer by prolonged dialysis during reduction and acylation or possibly to the presence of agglutinating activity in the γA-immunoglobulin class. This class may be of intermediate sedimentation rate and susceptible to reduction by 2-mercaptoethanol (27).

Sera with and without anti-Gm agglutinating activity, with high and low agglutinating titers, and with agglutinating activity outside the γM-immunoglobulin class were screened for precipitating antibody against various γ-globulin preparations without success.

*Development of anti-Gm activity.* It was possible to obtain repeated samples on several of those children who had no agglutinating activity but whose γ-globulin lacked one of the Gm factors under consideration. Patient M.D., a 15-year-old girl with thalassemia major, had received over 100 transfusions. Her serum phenotype was Gm(a + b + x −). Three serum samples obtained over a 2-month period in 1962 were without agglutinating activity even in the undiluted state. Nine months later anti-Gm(x) activity at a titer of 1:10 was present. No inhibition of the standard Gm(x) typing system could be detected by any of the samples from this patient, and it is therefore felt unlikely that transfused Gm(x +) γ-globulin could have masked the presence of anti-Gm(x) activity in the first three specimens.

*Anti-γ-globulin activity not specific for Gm factors.* In the course of these studies several sera were found that gave definite capillary precipitin reactions with heat-aggregrated human Fraction II. As this reaction was not considered typical of anti-Gm sera from clinically normal donors, the sera in this study were screened with the latex-Fraction II fixation test. The results are listed in Table II. Eighteen of the 65 children were found to have positive latex fixation tests at a titer of 1:20 or greater. It is apparent from the data in Table II that latex positivity correlated with the presence of anti-Gm activity and was generally found in those sera with the highest anti-Gm agglutinating titer. In addition, a suggestive correlation was found between the occurrence of a positive latex test and the number of transfusions received. Of those known to have received 50 or fewer transfusions, 15.4% were latex positive. The 51 to 100 transfusions group had 28.6% latex positives, 101 to 200 had 50% positives, and of

TABLE III

*The presence of agglutinating activity with and without Gm specificity in sera from repeatedly transfused patients*

| Source of serum | | | | Agglutination titer against human Rh(+) erythrocytes coated with | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Anti-D Rip* | | | | |
| | | | | | Agglutinator absorbed with | | | | |
| | | | | Anti-D 2368* Saline | | Gm(a −) γ-globulin (1 mg/ml) | | Gm(a +) γ-globulin (1 mg/ml) | |
| Patient | Diagnosis | No. transfusions | Specificity of agglutinator | | Saline | Unagg.† | Agg.† | Unagg. | Agg. |
| A.S. | "Normal" | Unknown | Anti-Gm(a) | 1:320‡ | 1:320‡ | 1:320‡ | 1:320‡ | 0§ | 0 |
| S.L. | Thalassemia major | >100 | Anti-Gm(a) | 1:40‡ | 1:320 | 1:160 | 1:20‡ | 1:10‖ | 0 |
| S.I. | Thalassemia major | >100 | Anti-Gm(a) | 1:80‡ | 1:320 | 1:320 | 1:80‡ | 1:40‖ | 0 |
| H.R. | Rheumatoid arthritis | None | Rheumatoid factor | 0 | 1:320 | 1:160 | 0 | 1:160‖ | 0 |

* Gm(a+x+) anti-D.
† Unagg. = unaggregated; agg. = aggregated at 63° C for 11 minutes.
‡ Gm(a) specific.
§ No agglutination by 1:5 dilution of agglutinator.
‖ Not inhibited by pooled, unaggregated human γ-globulin as Fraction II.

those who had received more than 200 transfusions 71.5% were latex positive. Some notable exceptions were found, however. Several sera with high titered anti-Gm(a) activity were repeatedly negative in the latex fixation test, and two sera had positive latex fixation tests but no detectable agglutinating activity. Three of these patients had received more than 200 transfusions and remained latex negative. Although many of the latex fixation titers were 1:320 or greater, in no instance did the degree of agglutination in any tube exceed 1 +, as compared to a maximal 4 + reading seen in rheumatoid sera with this test. All tests were reproducible, however, and essentially identical readings were obtained by two different observers on tests run at different times. None of the 52 children who had not been transfused and served as a control group were positive by this test.

Because these results suggested that anti-γ-globulin activity other than that specific for the Gm system was present in some of these sera, further studies of hemagglutinating systems were performed. A number of sera were screened simultaneously with two incomplete anti-D coats of comparable titer, one (2368) agglutinated infrequently by rheumatoid sera and the other (Rip.) recognized for its broad reactivity with rheumatoid factor (28). Both are of use as Gm(a) and (x) typing reagents when used with appropriate agglutinators. In most instances simultaneously run agglutinating titers against these two coats agreed within one twofold dilution. In some sera, however, agglutination titers against erythrocytes coated with anti-D Rip. were significantly higher than those against cells coated with anti-D 2368. The reverse situation did not occur. Inhibition studies done with two such sera (S.L. and S.I.), along with studies on one clinically normal donor (A.S.) who has an anti-Gm(a) agglutinator of comparable titer and with studies on a patient with rheumatoid arthritis (H.R.) whose serum had no detectable anti-Gm(a) activity, are presented in Table III. In all instances, inhibition with γ-globulin from a Gm(a −) individual was minimal or nonexistent, as was inhibition with Gm(a +) γ-globulin in the rheumatoid patient. In serum A.S. Gm(a −) γ-globulin after aggregration did not lower the Gm(a) specific agglutination titer, but in both transfused patients and in the rheumatoid, heat-aggregated γ-globulin even of Gm(a −) phenotype caused significant inhibition. Gamma globulin isolated from a Gm(a +) donor, or pooled human γ-globulin as Cohn Fraction II, which is Gm(a + b + x +) and presumably contains all Gm factors, totally inhibited agglutinator A.S. and only partially inhibited the remaining sera. Gm(a +) γ-globulin after aggregation totally inhibited all sera. Similar studies with one of these sera (S.I.) and Gm(a +) γ-globulin from three different donors gave identical results. The agglutinating activity that remained after absorption of this serum with aggregated Gm(a −) γ-globulin was totally inhibited by human Fraction II and all Gm(a +) sera.

*Studies in repeatedly transfused adults.* Sera from 85 adults who had received five or more transfusions were obtained from a variety of

sources. The average age of this group was 41 with a range from 18 to 72 years. The diagnoses included various types of malignancy, hemolytic anemia, trauma, gastrointestinal bleeding, hemophilia, and various surgical procedures. None of this group of patients had clinically recognized rheumatoid arthritis as far as could be determined. Although full details on transfusions in this group are not available, the majority of transfusions were apparently administered as whole blood, according to general information from physicians supplying the samples.

Twenty-eight sera from this group were found to have agglutinating activity against at least one of a panel of nine incomplete anti-D antibodies used as cell coats, although inhibition studies did not reveal Gm(a), (b), or (x) specificity in any of these agglutinating sera. Each serum was tested for its ability to agglutinate cells coated with at least six and at most nine different anti-D coats. As shown in Table IV approximately one-half of the various anti-D antibodies were agglutinated by the different agglutinating sera, one agglutinating a single anti-D and 15 agglutinating more than one-half of them. Two sera agglutinated all anti-D coats tested, and one serum agglutinated eight of nine. Among the 85 adults, 17 were found to have a positive latex fixation test, 13 occurring in sera that agglutinated anti-D coated red cells. In all but one instance maximal latex agglutination in any dilution was weak, but positive tests were reproducible and verified by two observers.

Comparison of those adults with and without hemagglutinating activity showed them to have received comparable numbers of transfusions (Table IV), although the hemagglutinating sera were from a patient group with a slightly higher mean age. Historical information on the adults in this study indicated that about half had received their transfusions over a relatively short period of time (2 years or less) and many in a period of weeks to months.

We have been unable to collect sera from an adequate number of patients comparable in age and diagnosis who had not been transfused to serve as a true control for the occurrence of antiglobulin factors in these adults. We have screened a random selection of 50 adults accepted as blood bank donors by the same tests. Six of the 50 had positive latex fixation tests by these criteria, and three of the 50 were able to agglutinate red cells coated with anti-D Rip.

## Discussion

The best explanation of the foregoing data is that humans may be immunized by the administration of isologous γ-globulin. In repeatedly transfused children, most of this immunization was based on antigenicity of genetic determinants of γG-globulin foreign to the recipient, and such anti-Gm activity, often of high titer, was found only when the patient's own γG-globulin lacked the genetic determinant involved. The 60% incidence of anti-Gm activity in this group contrasts with the less than 5% seen in our nontransfused controls. The incidence of anti-Gm activity in our control group is comparable to that reported by Ropartz and Lenoir (2) in the general population. Wilson and Steinberg have reported the incidence of anti-Gm antibodies as 11% between the ages of 6 months and 5 years and less than 4% above that age (29). Three-fourths of our patients with Gm-specific agglutinators were over 5 years of age. Our studies have indicated that, although immunization by maternal γ-globulin may

TABLE IV

*Anti-γ-globulin activity in sera from repeatedly transfused adults*

| Hemagglutinating activity | No. patients | Mean age | Mean no. transfusions | Fraction of Rh coats agglutinated* | No. latex positive |
|---|---|---|---|---|---|
| | | *years* | | | |
| Present | 28 | 45 ± 15† (19 – 72)‡ | 33 ± 37 (10 – 180) | 0.52 ± 0.24 (0.11 – 1.00) | 13 |
| Absent | 57 | 39 ± 15 (18 – 72) | 31 ± 34 (5 – 150) | 0 | 4 |

* No. Rh coats agglutinated/no. Rh coats tested.
† Standard error of the mean.
‡ Range.

have played a role in the appearance of some of the anti-Gm activity reported here, many if not most of these children were immunized only by γ-globulin administered postnatally. This was evident from analyses of maternal γ-globulin, which in many instances lacked the genetic factor to which antibodies were produced. Further confirmation for this concept came from the serial analyses that showed the development of anti-Gm(x) activity in a 15-year-old patient receiving multiple transfusions. Studies to date have suggested that most if not all antibodies resulting from immunization by maternally transferred γ-globulin appear within the first 2 years of life (11, 29). Blumberg, Bernanke, and Allison have reported the occurrence of precipitating antilipoprotein antibodies in a number of repeatedly transfused individuals (30). Some of the children in this study have also been studied by Blumberg, and comparison with his data on these children indicates that anti-γ-globulin activity does not correlate with the presence of either antilipoprotein or antileukocyte antibodies.

A correlation between the number of transfusions and the incidence of anti-Gm antibodies is suggested by the data presented here (cf. Table II) in that about 75% (six of eight) of the children who were known to have received more than 200 transfusions developed anti-Gm antibodies, whereas only about half (14 of 26) of those who had received 50 or fewer had these antibodies. Such data are difficult to interpret, however, unless corrected for differences in phenotypic composition of the transfusion groups. For instance, it is clear that anti-Gm(a) activity developed only in those children who were themselves Gm(a) negative. Of the six Gm(a) negative children who had received more than 200 transfusions in our series, five developed anti-Gm(a) antibodies (83%). Of the 13 Gm(a) negative children who had received 50 or fewer transfusions, 12 (92%) developed anti-Gm(a) antibodies. Such data as those presented by Vierucci (8) do not allow evaluation of this factor.

In looking at data on repeatedly transfused children one is impressed by the occurrence of individuals who are phenotypically "receptive" (that is, lacking one of the Gm factors) and who have received massive numbers of transfusions without developing a Gm-specific antibody. Such observations suggest that something in addition to antigenic mass is an important determinant in immunization against the Gm factors. Similarly, although single instances of anti-Inv activity and of combined Gm specific antibodies in repeatedly transfused children have each been reported (31, 8), such occurrences appear to be rare. It is known that the Gm(a) and Gm(x) factors are carried on the same γG-globulin molecule (32), and it is possible that the Gm(a) determinant is the more antigenic and more likely to elicit an antibody response. This is supported by the higher incidence of anti-Gm(a) than anti-Gm(x) activity in these children. The presence of anti-Gm(a) antibody may then inhibit response to the Gm(x) determinant, either due to rapid clearance of the (a + x +) molecules or steric interference with accessibility of the Gm(x) site. Individual variability in response, low antigenicity of the immunizing determinants, and variations in the Gm phenotype of the transfused blood also undoubtedly play a role.

The absence of anti-Gm activity in the repeatedly transfused adults is of interest. Most of the children, especially those with thalassemia major, have received transfusions at regular intervals of 1 to 3 months since the early months of life, frequently as packed red blood cells. Thus the exposure of this group as a whole has been at relatively regular and well-spaced intervals over a number of years, often occurring as small doses of isologous γ-globulin. Available information indicates that most of the adult patients had received their transfusions as whole blood often given in a relatively short period of time. This suggests that perhaps the schedule of injection offers the major difference in results of immunization between the two groups. Stiehm and Fudenberg (33), studying children who had received a single massive antigenic exposure as exchange transfusion at birth, found a high incidence of hemagglutinating anti-γ-globulin activity (34%), but only an 11% (3 of 26) incidence of Gm-specific agglutinators. As 12 of the 17 patients in their study on whom ages are given were 5 years of age or younger, the significance of this finding when contrasted with the incidence of Gm-specific antibodies in the general population this age is unclear (29). That age of the recipient influences the development of anti-Gm antibodies

after appropriate antigenic exposure remains, however, a definite possibility.

Certain antigens such as typhoid O have been shown to elicit primarily if not exclusively a γM-immunoglobulin response in the human (34). Isoantibodies to various red blood cell antigens, however, may be found within all three of the immunoglobulin classes (35, 36). Our data clearly indicate that most of these anti-Gm isoagglutinators are γM-globulins, although in two instances at least part of the agglutinating activity was not in this class. The occurrence of anti-Gm activity in other than the γM-globulins has been reported (37).

Anti-γ-globulin activity in addition to that with Gm specificity was apparent in the repeatedly transfused children and has been noted by others (8, 33). This anti-γ-globulin activity had certain characteristics in common with rheumatoid factor in its ability to agglutinate cells coated with a broadly specific incomplete anti-D, its ability to give visible reaction with heat-aggregated human γ-globulin, and its absorption by aggregated γ-globulin unrelated to the Gm phenotype. It was clear that this activity could be completely absorbed without removing Gm-specific hemagglutinating activity. This is in contrast to the anti-Gm agglutinators associated with the more classical form of rheumatoid factor, which are absorbed along with the "nonspecific" anti-γ-globulin factors by Gm-negative heat-aggregated γ-globulin (38). This difference was also apparent in the weak agglutination of Fraction II coated latex particles, even when high titers of such activity were present. It has been suggested (8) that anti-γ-globulin activity occurring in the serum of repeatedly transfused children represents a new class of agglutinators. Data presented here, however, indicate that anti-Gm antibody activity in repeatedly transfused children resides with different γ-globulin molecules than those carrying the "non-Gm" anti-γ-globulin activity; such antibodies are apparently identical to anti-Gm antibodies found in nonrheumatoid, nontransfused individuals (monovalent, isospecific, mainly γM-globulin antibodies whose activity is not absorbed by heat-aggregrated γ-globulin lacking the appropriate Gm factor). Their occurrence in association with the non-Gm-specific anti-γ-globulin activity is probably a function of the unique situation offered by repeated transfusions.

The nature of this non-Gm anti-γ-globulin activity is not clear, however. The correlation between its presence and the number of transfusions received suggests that it is probably related to the receipt of isologous γ-globulin, but the possibility that in some instances autoimmunization by autologous antibody reacting with the transfused red cells cannot be excluded. This activity is probably not due to antibody against an unidentified Gm determinant. At least, it was not susceptible to absorption by unaggregated pooled human γ-globulin as Fraction II. In many ways it is similar to the classical form of rheumatoid factor, as judged by the various serological parameters mentioned above. Conceivably this activity could result from immunization by aggregated or partially denatured γ-globulin occurring in the transfused preparations. It is of note that none of the children in this study had clinical evidence of rheumatoid arthritis.

About one-third of the adults who had received multiple transfusions were found to have anti-γ-globulin activity demonstrable in hemagglutination tests. The significance of this finding is unclear in the absence of an adequate control group. It is well recognized that age has a marked influence on the occurrence of rheumatoid factor activity in the normal population (39). Waller, Toone, and Vaughan (40) have reported positive tests using anti-D coated human red cells in about 17% of individuals 60 years or older. Below age 60, however, this test was positive in no more than 4% of their normal population, and they noted no increased incidence of positive latex fixation tests in 141 patients with osteoarthritis and nonrheumatic diseases. The occurrence of rheumatoid factor in the presence of a variety of nonrheumatic diseases is, however, well recognized (41–46). It is of possible significance that 14 of the 20 repeatedly transfused adults whose ages are known and who had positive hemagglutination tests in this study were less than 60 years old. In most the known diagnoses were not those classically associated with the presence of rheumatoid factor. Two of these agglutinators, although low in titer, were active against all and one against eight of the nine anti-D antibodies with which they were tested, suggesting a similarity in such instances to "anti-antibody" as described by Milgrom, Dubiski, and Wozniczko (47). We have not been able to

assess other characteristics of rheumatoid factor, such as cross-reactivity with rabbit γ-globulin, as they might occur in these sera due to the small quantities of material available. Such studies will undoubtedly shed further light on the relationship between the non-Gm–specific anti-γ-globulin activity found in these repeatedly transfused patients and classical rheumatoid factor as seen in patients with rheumatoid arthritis.

## Summary

Studies on sera from 65 children and 85 adults who had received repeated doses of γ-globulin in the form of blood transfusions revealed that 60% of the children and 30% of the adults had some form of antibody against γ-globulin. In children, the predominant anti-γ-globulin specificity was for one of the genetic (Gm) factors, although non-Gm specific, rheumatoid factor-like activity was also demonstrated. The thesis that these Gm-specific antibodies arose by immunization with transfused, genetically foreign γ-globulin is supported by *1*) the occurrence of anti-Gm specificity only against a Gm factor absent in the patient's γ-globulin phenotype, *2*) the high incidence of this activity compared to a nontransfused, control population, *3*) the development under observation of anti-Gm activity in one patient at the age of 15 years, and *4*) the frequent absence of the immunizing Gm factor in the maternal γ-globulin. In adults, hemagglutinating antibody directed against a number of human incomplete anti-D antibodies was found. This could have arisen from immunization by denatured or aggregated γ-globulin in the transfused preparations or by immunization with autologous γ-globulin present as antibody against transfused blood cells ("anti-antibody").

These studies have documented the antigenicity of isologous γ-globulin for man and have demonstrated that Gm-specific antibodies and rheumatoid factor-like activity may arise by immunization with isologous γ-globulin. They offer evidence as to the origin of antibodies specific for the Gm factors of human γ-globulin occurring in clinically normal individuals and are compatible with the concept that anti-γ-globulins occurring in patients with rheumatoid arthritis are antibody in nature.

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