The etiology of the rheumatoid factors in patients with rheumatoid arthritis and of anti-globulin antibodies in normal individuals remains obscure despite recently documented observations. Allen and Kunkel (1963) and Vierucci (1965) have reported an increased incidence of anti-globulin antibodies in children who received multiple transfusions for chronic anemia. Fudenberg et al. (1964) and Stiehm (1965) found that children who had been receiving repeated γ-globulin injections were more prone to produce anti-globulin antibodies than children not exposed to γ-globulin. These data furnish indirect evidence of the isoantigenicity of genetic determinants in human γ-globulin. From the clinical standpoint, the observations are of interest to pediatricians administering γ-globulin to children whose sera show low levels of γ-globulin, and whose clinical courses are characterized by repeated or chronic infections.

This study was undertaken to determine the incidence of anti-globulin antibodies in non-hospitalized children with chronic or recurrent upper respiratory infections, only some of whom had received γ-globulin injections.

Materials and Methods

The 185 children studied were under the care of their private physicians or attended the pediatric clinic. Hospital patients were not included. With the cooperation of the participating physicians, history forms were completed on each child, recording age, race, sex, the amount and duration of globulin therapy, reason for administering γ-globulin, transfusion history, and whether or not joint symptoms were present. All children with a transfusion history were excluded from this study in order to obtain a more uniform population.

The 44 children who had received γ-globulin injections had γ-globulin levels less than 500 mg per 100 ml. The duration of γ-globulin therapy reflected to some degree the course and duration of their respiratory infections. The 141 children who had "never" received any γ-globulin comprised the largest group. Although these children were recorded as "never" having had γ-globulin, it has been customary for some physicians to give a small amount of γ-globulin with measles vaccine. Some of these 141 children were tested before the initiation of γ-globulin therapy. Thus, these children do not necessarily represent a group of children with normal levels of γ-globulin.

All sera were tested at a 1:20 dilution by: the slide latex test (Hyland), the sensitized human cell test, and the sensitized sheep cell test. These tests have been described previously (Waller et al., 1961). In addition, most of the sera were tested at a 1:5 dilution with selected sensitized cells known to detect anti-Gm* Gmβ, Gmx and Gmf activity.

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Results

Anti-globulin Antibodies

Of the 185 children studied, only 44 had received injections of γ-globulin at the time of testing. All of these children were tested at a 1:20 dilution of their serum with the slide latex test and the sensitized human cell test. The latex test was strongly reactive in only one child and weakly reactive in 12 children. Only one of these 13 children had received γ-globulin injections. The human cell test was positive in eight children (4.3%), only one of whom had received γ-globulin. This incidence does not differ significantly from that characteristic of the normal adult population (Waller et al., 1964). The sensitized sheep cell test (Waaler-Rose Test) was performed on 135 children, including all children found to be positive by any of the other tests. In no instance was a positive sheep cell test observed.

Since the titers of anti-globulin antibodies in normal individuals may often fall below 1:20, 140 of the original 185 children were tested at 1:5 dilution of their serum with Ripley sensitized cells. Eighteen children (12.9%) gave positive tests at this dilution. However, of these 18 children with positive tests, only three had received injections of γ-globulin.

Thus, to look at the results in another way, of the 108 children who had not received injections of γ-globulin and whose sera were tested at a 1:5 dilution, 15 (13.9%) gave positive tests. However, of the 32 children who had received γ-globulin and whose sera were tested at a 1:5 dilution, only three (9.4%) gave positive tests. These differences are probably not statistically significant (p > .4).

Figure 1 compares the titers of anti-globulin antibodies in children who had received γ-globulin injections with those who had not received any γ-globulin. The left bars, 1:5, represent the negative group in our study, since the children were not tested at a level below 1:5. Ten children gave titers of 1:5, but only two of these had had γ-globulin. Eight children had titers above 1:5, but only one child had had γ-globulin. Thus, among the 44 children who had received injections of γ-globulin, only three children gave positive tests for anti-globulin antibodies. There was no correlation between the amount of γ-globulin administered, the duration of globulin therapy, and the presence of a positive test for anti-globulin antibodies. Three children had had in excess of 100 ml of γ-globulin over a period from one to three years, and all gave negative tests at 1:20 dilution of their serum. Two of the three were also negative at 1:5 dilution of their serum.

Gm Specificity of Anti-globulin Antibodies

In addition to the sensitized human cell test (Ripley), four other anti-D sera were used to sensitize cells for Gm specificity of the children's sera. Ninety children were tested for Gm specificity. Of these 90 children, 10 agglutinated the Ripley sensitized cells and five of the 10 agglutinated one or more of the other sensitized cells. Of these 10 children, five gave specific anti-Gm activity, one anti-Gm(f), one anti-Gm(x), and three anti-Gm(a). The sera of the other five children agglu-
tinated only the Ripley sensitized cells, and it was not possible to obtain Gm specificity. Of the five children whose sera showed specific anti-Gm activity, only one had received γ-globulin.

Lack of Correlation between Presence of Anti-globulin Antibodies and Level of γ-globulin or Age of Children

There was no apparent correlation between the presence of hypogammaglobulinemia (<500 mg per 100 ml) and the presence of anti-globulin antibody. There was also no correlation between the ages of the children and the presence of anti-globulin antibody. Among the children up to three years of age, 12% gave positive tests for anti-globulin antibody at a 1:5 dilution of their sera. Among the children over three years of age, 13.7% gave positive tests. Of the 185 children studied, four children gave history of joint complaints, none had had γ-globulin at the time of the study, and none had anti-globulin antibodies demonstrable in their sera.

Injection of γ-Globulin into Adults

We have studied the effects of subcutaneous injections of pooled γ-globulin in four young healthy male volunteers. These men were all Gm(a−) and received (.5 ml) injections of pooled γ-globulin weekly. In addition to tests for anti-globulin antibodies, slide latex tests, and sensitized sheep cell tests, the following tests were performed: serum electrophoretic pattern, sedimentation rate, hemoglobin, and total protein.

Two of the volunteers received a total of eight injections of γ-globulin over a period of three months and two received 16 injections over a period of one year. In no instance did these four individuals produce anti-globulin antibodies, nor were any changes noted in the other tests performed.

Discussion

Although the incidence of anti-globulin antibodies in children receiving multiple transfusions is markedly increased, we were not able to demonstrate this increased incidence in the children receiving pooled γ-globulin. There are several possible explanations for these observed differences. The children receiving multiple transfusions had a definite disease entity while the children that we studied were “normal” except for chronic or recurrent upper respiratory infections. The evaluation of just how chronic, recurrent, or severe the infection rested with the individual physician; however, all of the children studied had electrophoretic patterns of their serum performed as a consequence of the degree or duration of their infection. No attempt was made in this study to separate the children according to degree or duration of their illnesses. Certainly, the children were not ill enough to require hospitalization during the period of this study.

It is possible that plasma is more antigenic than pooled γ-globulin or that the globulin administered intravenously is a better antigen than when it is administered intramuscularly. In addition, the children receiving multiple transfusions received more plasma for a longer period of time than did the children receiving pooled γ-globulin.

Stiehm and Fudenberg (1965) studied 14 hypogammaglobulinemic children who had received many γ-globulin injections and found that nine of these children (64.3%) had developed anti-globulin antibodies. Among the 24 untreated siblings of these children, only two agglutinators were found (8.3%). We are not able to explain the discrepancies in these results other than by the fact that we did not use as controls the siblings of the hypogammaglobulinemic children. However, in addition, one may presume that the untreated siblings were untreated because they were not hypogammaglobulinemic or that they were not subject to chronic or recurrent infection. Some of our untreated children were hypogammaglobulinemic and most were subject to chronic infection. After the testing of their sera, some children were started on γ-globulin therapy.

Since an equal number of our children who had not received γ-globulin produced anti-globulin antibodies, pre-
sumably other, as yet unknown, agents are as likely to stimulate the production of these antibodies as is the administration of pooled γ-globulin.

Steinberg and Wilson (1963) studied eight non-transfused donors of γ-globulin antibodies and found that in each instance the donor’s mother had the globulin factor that the donor lacked. They assumed that the donor’s antibody was formed as a result of transplacental isoimmunization. However, we have been unsuccessful in our attempts to elicit anti-globulin antibodies in normal adult Gm(a−) volunteers by the injection of pooled γ-globulin.

There is no definitive evidence that the serological reactions called rheumatoid factors in patients with rheumatoid arthritis and anti-globulin antibodies in normal individuals, differ except in strength of reaction (titer) and possibly in specificity. Not all investigators are convinced that the idea of an antigen-antibody relationship for γ-globulin (Gm) and anti-globulin reagents (anti-Gm) in man is firmly established (Grubb, 1961). Certainly, the deleterious effects of the serological reactions called “rheumatoid factors” have not been proven at the present time. Regardless of these unresolved questions, it is not established that the isoantigenicity of γ-globulin is such as to override its therapeutic potential in infection.

Summary

1. We examined sera from 185 non-hospitalized children, 44 of whom had received repeated injections of γ-globulin for upper respiratory infections.
2. There was no significant difference in the incidence of anti-globulin antibodies in the two groups.
3. The presence of anti-globulin antibodies could not be correlated with the level of γ-globulin in the children’s sera or with the age of the children.

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References


