Transfusion and Alloimmunization in Sickle Cell Disease


In 1,814 patients with sickle cell disease who had been transfused, the overall rate of alloimmunization to erythrocyte antigens was 18.6%. The rate of alloimmunization in this group appears to be an explicit function of the number of transfusions received because it increases exponentially with increasing numbers of transfusions. Alloimmunization usually occurred with less than 15 transfusions, although the rate of alloimmunization continued to increase when more transfusions were given. The rate of alloimmunization was less in patients with hemoglobin SC disease and sickle-β-thalassemia because these patients had received fewer transfusions. Children less than 10 years old had a slightly lower rate of alloimmunization than patients in other age groups even after correction for the number of transfusions given. Women were more frequently alloimmunized than men; this was largely due to the fact that women received more transfusions than men, but in the age group 16 to 20 years the increase may have been due in part to alloimmunization owing to pregnancy. Forty-five percent of those alloimmunized made antibodies of only one specificity; 17% made four or more antibodies reacting with different antigens. Antibodies to the C and E antigens of the Rh group, the Kell antigen, and the Lewis antigens were most commonly made. These findings may be important in formulating a rational transfusion policy in sickle cell disease.

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Transfusion of red blood cells (RBCs) is a common practice in the treatment of patients with sickle cell disease (SCD) for two reasons: (1) the infusion of erythrocytes increases the oxygen-carrying capacity of the blood in the anemic patient, and (2) the replacement of the abnormal RBCs with normal ones may alleviate the symptoms or prevent the complications of the disorder. The practice of transfusion is so common that most adult patients with SCD have been transfused at one time or another, many with a great number of units of blood.

One of the major complications of transfusion is alloimmunization in the recipient to antigens present in the transfused blood. We will review the experience of the participants in the Cooperative Study for Sickle Cell Disease (CSSCD) in the transfusion of blood to patients with sickle cell disease. In particular, we will analyze the rate of alloimmunization as a function of age, sex, hemoglobin phenotype, number of transfusions, and antigenic differences.

MATERIALS AND METHODS

Patient population. All 3,047 patients included in this study were among 3,821 patients who were enrolled in the CSSCD between March 1979 and September 1984. The criteria for inclusion and recruitment have been described previously.

The diagnosis of SCD (Hb SS), sickle cell-β-thalassemia (β- or β0), or hemoglobin SC disease was confirmed by the Centers for Disease Control (CDC) (Atlanta GA), except for 48 patients who were transfused at entry and 68 patients who did not have confirmatory studies performed for various reasons. The diagnosis of hemoglobin S-β0 thalassemia was confirmed in most cases by globin chain synthesis studies.

Data collection. Data were acquired by standardized methodology using forms that were completed during the care of the patient. The incidence of transfusion before entry into the study was determined by retrospective recall of the patient. Transfusions administered during the conduct of the study were recorded on forms that were submitted after each transfusion. The prevalence and incidence of alloantibodies was determined in two ways:

(1) Samples of serum were collected at entry on all patients with a history of transfusion and 1 to 2 months after transfusion on all patients transfused during the study. These were analyzed for alloantibodies to RBC antigens (using standard blood bank techniques) at a central laboratory located at Duke University (Durham, NC) and at the transfusion service laboratories at the various clinics participating in the study.

(2) The transfusion records of all patients who were transfused before entry or during the study were examined for analysis of alloantibodies in serum to RBC antigens.

In preliminary studies, the results of these two methods of acquiring data were found to be concordant in about 90% of cases, and the data from both were used in analysis.

Data were submitted to the CSSCD Statistical Coordinating Center at the University of Illinois at Chicago. Scientific Information Retrieval (SIR), an integrated database management system and SAS, a statistical software package (SAS Institute, Cary, NC), were used for data management and analysis.

Definition of alloimmunization. Patients were said to be alloimmunized if antibodies to RBC antigens were identified during the study (see Table 1 for list). Patients whose serum contained other designated antibodies such as atypical, autocontrol, anti-I, high titer, low avidity (HTLA), anti-HLA, etc were considered not to be alloimmunized to erythrocyte antigens.

In some analyses, antibodies were divided into two groups: those that may arise only with pregnancy or transfusion, and those that may occur without transfusion or pregnancy but may be generated by those events; the latter are italicized in Table 1.

Statistical methods. Comparisons of demographic characteristics and transfusion and alloimmunization prevalence rates between groups were made using chi-squared tests. Mantel-Haenszel25 and

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the data were grouped and only the midpoint value of the group was used (0, 5 [1 to 9], 30 [10 to 50], and 75 [greater than 50]).

Transfusion rates were assessed using log-rank statistics to corroborate these results. Differences in sensitization rates between age, sex, transfusion history, and pregnancy groups were assessed using exponential models, which appeared to fit the data well. Log-rank statistics were used to assess differences in sensitization rates between age, sex, transfusion history, and pregnancy groups while controlling for the number of transfusions administered before entry.

To assess differences in the methodology of data collection, the prevalence of transfusion and alloimmunization at entry was analyzed separately from their incidence during the study.

RESULTS

Age, sex, and hemoglobin phenotypes. The distribution by hemoglobin phenotype and age at entry of the participants in the study are shown in Table 2. Fifty-three percent of the participants were female; this proportion did not vary significantly in any of the age or phenotype groups.

Rates of transfusion and alloimmunization. Because of differences in the methodology of data collection, the prevalence of transfusion and alloimmunization at entry was analyzed separately from their incidence during the study.

Transfusion prevalence rates at entry. The age, hemoglobin phenotype, proportional distribution within entry age group, and alloimmunization status of patients who had been transfused before entry into the study are shown in Fig 1. There was a significant difference in the percentage of females transfused (52%) compared with the percentage of males transfused (47%) (P = 0.004). The proportion of patients who had been transfused was significantly smaller in the group of patients with a diagnosis of Hb SC or HbS/β+ thalassemia than in the group of HbSS or HbS/β0 thalassemia (P < 0.001).

There is a significant increase in the percentage of patients transfused with increasing age (P < 0.001). This trend is seen in each genotype.

Prevalence of alloimmunization at entry. Prevalence rates of alloimmunization at entry are shown in Fig 1 for study patients stratified by age and hemoglobin phenotype. The difference between the proportion of HbSS patients alloimmunized (13.1%) and the proportion of non-HbSS patient alloimmunized (9.1%) was not significant (P = 0.07). The prevalence rate of alloimmunization in patients entered at less than 10 years was significantly lower than that of patients entered at ≥10 years (P = 0.009). The prevalence of alloimmunization was significantly greater in females than in males (P = 0.01); this was largely because of differences in the age group 10 to 19 (P = 0.01).

Incidence of transfusion and alloimmunization during the study. Of 3,047 patients studied, 1,044 were transfused during the course of the study; 741 (71%) of these 1,044 patients had a history of transfusion before entry. One hundred thirty-six (18%) of the 741 patients transfused before entry were alloimmunized at entry (103 of these 136 had been alloimmunized to at least one transfusion-related RBC antigen as defined above, and 33 to RBC antigen(s) alloimmunization to which may occur in the absence of transfusion). Of the 605 patients transfused but not alloimmunized before entry, 94 (15.5%) developed antibodies after on-study transfusion; alloantibodies were identified in 55 (18.2%) of the 303 patients who had not been transfused before entry.

Table 2. Age and Hemoglobin Phenotype of Patients Taking Part in the Transfusion and Alloimmunization Study

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Age at Entry (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;2</td>
</tr>
<tr>
<td>Hemoglobin SS</td>
<td>277</td>
</tr>
<tr>
<td>Hemoglobin SC</td>
<td>123</td>
</tr>
<tr>
<td>Hemoglobin S-β+ thalassemia</td>
<td>18</td>
</tr>
<tr>
<td>Hemoglobin S-β0 thalassemia</td>
<td>9</td>
</tr>
<tr>
<td>Uncertain</td>
<td>49</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td>476</td>
</tr>
</tbody>
</table>

Fifty-three percent of the patients were female and the proportion did not vary significantly in any age or hemoglobin phenotype group.
Of the 1,044 patients transfused while on study, 673 were included in the analysis of incidence of alloimmunization to transfusion-related RBC antigens; 640 were included in the analysis of incidence of alloimmunization to any RBC antigen. Patients were excluded from the incidence analyses if: (1) they were alloimmunized before their first on-study transfusion; (2) the number of transfusions before alloimmunization was unknown; or (3) an alloantibody record for nonalloimmunized patients was not available after any on-study transfusion.

Hemoglobin phenotype and age of patients included in the analysis of incidence of alloimmunization to transfusion-related RBC antigens and the rate of alloimmunization in each group are given in Fig 2. The proportion of males and
females included in this group was not significantly different from the proportion in the study as a whole, and the proportion of females alloimmunized was not significantly different from that of males ($P = .33$). The proportion of HbSS patients alloimmunized was significantly higher than that of non-HbSS patients alloimmunized ($P = .001$). The difference in alloimmunization rates between patients whose first transfusion was at less than 10 years of age (9.6%) and patients whose first transfusion was at $\geq$10 years of age (20.7%) was statistically significant ($P < .001$).

To express the relationship between incidence of alloimmunization and number of on-study transfusions, exponential survival curves were fitted to the data. The exponential model fitted the data well, indicating that the chances of sensitization continue to rise with increasing number of transfusions. Figure 3 portrays the fitted curves based on the data from the “purest” subgroup of patients included in the analysis: ie, the 238 patients who were not transfused before entry. Similar curves were derived when the data from all 673 patients were included in the analysis.

Survival function techniques described in Materials and Methods were used to see whether sex, age at time of first on-study transfusion, number of transfusions before entry (categorized as 0, 5, 30, or 75), or hemoglobin phenotype influenced rates of alloimmunization while controlling for the number of units of blood received while on study. Proportional hazards regression results indicated that only the main effect of age noted above was significant ($P = .005$); patients whose first on-study transfusion was at less than 10 years of age appeared to require more transfusions to become sensitized than patients whose first on-study transfusion was at $\geq$10 years of age. Analysis of incidence of alloimmunization to any RBC antigen yielded similar results.

Antigens against which alloantibodies are made. The specificity of the 765 antibodies identified in 338 patients either at entry or during the course of the study is presented in Table 1. The incidence of each specificity was analyzed for males and females and for each phenotype; no significant differences were found in these comparisons, and the figures given are for both sexes and all phenotypes.

Prevalence of multiple antibodies. The frequency distribution of the number of antibodies of different specificity identified in study subjects is shown in Table 3.

Persistence of antibodies. Because antibodies may become undetectable with the passage of time from the alloimmunizing event, the persistence of 205 alloantibodies (26.8% of all identified) for which follow-up data were available was analyzed. To be included in the analysis, an antibody identified on an initial examination had to have been tested for again on two subsequent occasions more than 365 days later. If the antibody was found on both subsequent tests, it was said to be persistent; if it was not detected on one or the other such subsequent test, it was said to be transient. The results are shown in Table 4. Although the rate of “transience” varies for different antibodies, approximately 35% of all antibodies in the analysis were not detectable on at least one of two subsequent occasions.

![Fig 3.](#)
DISCUSSION

In the usual practice of transfusion of blood, alloimmunization is limited by several factors. Many of the antigens present on erythrocytes infrequently give rise to alloimmunization even when injected into patients lacking the antigen; the rate of immunization from transfusion ranges from 70% for the Rh(D) antigen to as low as 0.5% for the Duffy antigens. The reasons for this variability in the immunogenicity of different antigens is not known.

The ability to react to alloantigens varies greatly from person to person. Some individuals will not become immunized to any antigens despite repeated transfusion, whereas others will become immunized, when transfused, to many of the antigens that they lack. In animals, the ability to respond to a foreign antigen is determined by genetic loci in the major histocompatibility locus (the so-called “immune response genes”). These genes have not been definitively identified in humans.

A low rate of alloimmunization in transfusion practice may be expected when there is homogeneity with respect to the erythrocyte antigens of the population providing blood and that receiving the blood. When the distribution of antigens is different in the population donating the blood and the population receiving the blood, greater alloimmunization may be expected among the recipients.

The present study is the first prospective evaluation of the rate of alloimmunization to RBC antigens in patients with SCD. We have analyzed the prevalence and the incidence of alloantibodies in a large series of patients of all ages and hemoglobin phenotypes.

The data probably represent a minimal estimate of the rate of alloimmunization because antibodies do not necessarily persist until recognition. In the present study, up to 37% of the antibodies that were detected at one time were not detected on subsequent testing. In studies with more frequent follow-up, a high incidence of anti-Lu" was noted shortly after transfusion; this antibody disappeared in all cases within a few months of its appearance. In the present study, anti-Lu" was not reported. Although frequent follow-up was mandated, it is still possible that patients may have had transient anti-Lu" alloantibodies.

The pattern of alloimmunization seen in this study may be compared with that seen in other studies of patients with SCD in which the rate has varied from 7.5% to 36%. The higher rates reported are generally in older and more highly transfused patients.

We have found that the primary determinant of the risk of alloimmunization is the number of transfusions (units) that are administered. The rate of alloimmunization for different age groups and different hemoglobinopathies closely parallels the proportion of patients who have received transfusions. Proportional hazards regression analysis and log-rank tests indicated that the rate of alloimmunization was not influenced by hemoglobin phenotype or sex per se when the number of transfusions administered was controlled. However, rates did appear to be influenced somewhat by age: the rate of alloimmunization in patients whose first transfusion was at less than 10 years of age was less than expected for the number of transfusions administered.

The data were analyzed separately for antigens related to pregnancy or transfusion and for all antigens; the same conclusions could be drawn in both analyses.

In several previous studies, it has been suggested that the rate of alloimmunization was greater for women than for men. In the present study, a slight difference was found in the overall rate of alloimmunization (16.2% for men vs 20.7% for women). This was seen to be a statistically insignificant difference when the number of transfusions was taken into account.

It has been suggested that the risk of primary alloimmunization decreases dramatically or ceases after 10 to 20 transfusions have been administered. This is interpreted to mean that with that number of transfusions, all who are...

<p>| Table 3. Prevalence of Multiple Antibodies of Different Specificity in Patients With SCD |
|-------------------------------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>No. of Alloantibodies Identified</th>
<th>Total N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1,476</td>
<td>81.4</td>
</tr>
<tr>
<td>1</td>
<td>152</td>
<td>8.4</td>
</tr>
<tr>
<td>2</td>
<td>94</td>
<td>5.2</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>1.8</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>1.4</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>0.7</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>0.4</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>0.3</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>0.2</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>0.1</td>
</tr>
<tr>
<td>≥10</td>
<td>2</td>
<td>0.1</td>
</tr>
<tr>
<td>Total</td>
<td>1,814</td>
<td></td>
</tr>
</tbody>
</table>

<p>| Table 4. Incidence of Nonpersistent Alloantibodies |
|-------------------------------------------|----------|----------|</p>
<table>
<thead>
<tr>
<th>Specificity</th>
<th>Incidence</th>
<th>Nonpersistent*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-D</td>
<td>44</td>
<td>5</td>
</tr>
<tr>
<td>Anti-C</td>
<td>102</td>
<td>11</td>
</tr>
<tr>
<td>Anti-E</td>
<td>143</td>
<td>16</td>
</tr>
<tr>
<td>Anti-V</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Anti-c</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Anti-M</td>
<td>26</td>
<td>3</td>
</tr>
<tr>
<td>Anti-S</td>
<td>31</td>
<td>2</td>
</tr>
<tr>
<td>Anti-K</td>
<td>95</td>
<td>11</td>
</tr>
<tr>
<td>Anti-Fy</td>
<td>62</td>
<td>8</td>
</tr>
<tr>
<td>Anti-Jk</td>
<td>36</td>
<td>3</td>
</tr>
<tr>
<td>Anti-Le</td>
<td>83</td>
<td>14</td>
</tr>
<tr>
<td>Anti-Le</td>
<td>42</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>682</td>
<td>78</td>
</tr>
</tbody>
</table>

* A nonpersistent antibody is one that was not detectable on two occasions at a time greater than 1 year after initial detection.
going to become alloimmunized have done so. This does not appear to be the case from the present data. As shown in Fig 3, the rate of alloimmunization is greatest with the first transfusions but continues to increase even with up to 50 transfusions. This is consonant with the data of Reisner et al., who showed in polytransfused patients with SCD that the risk of alloimmunization to RBC or tissue antigens continues even after 100 or more units of blood are administered.

It is difficult to compare the rate of alloimmunization to RBC antigens in SCD with that in other diseases. The estimated rate in patients with thalassemia has generally been about 5%,”31,32; patients with this disease are generally transfused to at least the same extent as are patients with SCD. However, these patients are generally transfused at an earlier age than are sickle cell patients, and it has been suggested that those transfused before age 3 had less alloimmunization than those whose transfusions began after that age.33

The rate of alloimmunization to RBC antigens has been estimated at 8% to 12% in patients with other diseases13,14; patients with hemoglobinopathies were included in these studies, but the rate of alloimmunization did not appear to be different for them. The careful prospective study of Lustumbo et al.10 found a much higher rate among patients receiving transfusions as part of cardiac surgery; however, when the figures are corrected for transient antibodies (chiefly anti-LuA1), cold agglutinins, and nonspecific antibodies, the rate of alloimmunization was 11.0%. It is clearly difficult to compare groups different in underlying disease, age, and number of transfusions. Nevertheless, from the data given, there is a strong suggestion that the rate of alloimmunization in SCD is greater than in most other groups.

The specificity of the antibodies found in this study are similar to those found in other studies of alloimmunization in SCD.12,14,17 Comparative data from populations other than those with thalassemia are not available, as it would be difficult to find age-matched controls that had received approximately the same number of transfusions. However, the relatively high rate of alloimmunization among patients with SCD could result in part from the differences in the incidence of some of the antigens in the African-American population (the recipients in this case) and the European-American population (the majority of donors in most institutions). Because a number of antigens are significantly less common in the African-American population,28 recipients in this population are at increased risk of alloimmunization if transfused with blood from donors of non-African origin.28 The calculated rate of alloimmunization was about twice as great for interracial as for intraracial transfusion to African-American recipients. These studies were predicated on the basis of single transfusions; in the present study we have shown that the chances of alloimmunization are increased with increasing number of transfusion. If these are consistently from a European-American donor population, the effect of the differences in antigenic composition in the two populations are probably magnified.

Others have suggested more extensive typing of donor RBCs to eliminate transfusion of cells bearing antigens that the recipient lacks.14 When a group of patients with SCD were given blood matched for 17 RBC antigens, the incidence of alloimmunization was reduced 10-fold.18 Determination of the Rh and Kell antigens was found to be useful in preventing alloimmunization in patients with thalassemia.35,36 Extensive antigen typing may be expensive and was not recommended in other studies.13,37 Nevertheless, typing only for the antigens commonly causing alloimmunization (C, E, K, etc) is practical and not expensive.

A similar effect may be achieved by increasing the pool of African-American donors of blood for patients with SCD or by voluntary directed donations by these donors to sickle cell patients. Studies in several centers are underway to determine if this is a feasible alternative in reducing the rate of alloimmunization among patients with sickle cell disease.

APPENDIX

The Cooperative Study of Sickle Cell Disease is funded by the Sickle Cell Disease Branch of the Blood Division of the National Heart, Lung, and Blood Institute. The following are cooperating clinics and senior investigators in the study:

Clinical Centers: Alta Bates Hospital, Berkeley, CA: Dr Robert Johnson; Boston City Hospital, Boston, MA: Dr Lillian McMahon; Children’s Hospital, Boston, MA: Dr Orah Platt; Children’s Hospital, Philadelphia, PA: Drs Kwaku Frempong and Frances Gill; Children’s Hospital, Oakland, CA: Drs Elliott Vishinsky and Bertram Lubin; Children’s Hospital National Medical Center, Washington DC: Drs Gordon Bray, John F. Kellehr, and Sanford Leiken; Columbia Presbyterian Hospital, New York, NY: Drs Arthur Bank and Sergio Piomelli; Duke University, Durham, NC: Drs Wendell F. Rosse and Thomas R. Kinney; George Washington University, Washington, DC: Dr Lawrence Lessin; Harlem Hospital, New York, NY: Drs Jeanne Smith and Yusaf Khakoo; Howard University, Washington, DC: Drs Roland B. Scott and Oswaldo Castro; Interfaith Medical Center, Brooklyn, NY: Drs Harvey Doak, Steven Diamond, and Rita Bellevue; LeBonheur Children’s Hospital, Memphis, TN: Drs Winfred Wagner and Judith Willimas; Medical College of Georgia, Augusta: Dr Paul Milner; State University of New York, Downstate Medical Center, Brooklyn: Drs Audrey Brown, Scott Miller, Ronald Rieder, and Peter Gillette; San Francisco General Hospital, San Francisco, CA: Drs William Lande, Steven Embury, and William Mentzer; St Luke’s Hospital, New York, NY: Drs Doris Wethers and Ranjeet Grover; University of Illinois, Chicago: Drs Mabel Koshy and Nasrin Talibshy; University of Miami, Miami, FL: Drs Charles Pegelow and Panpit Klug; University of Mississippi, Jackson: Dr Martin Steinberg; University of Tennessee, Memphis: Dr Alfred Kraus; Washington University, St Louis, MO: Dr Harold Zarkowsky; Wyley Children’s Hospital, Chicago, IL: Dr Carlton Dampier; Yale University, New Haven, CT: Drs Howard Pearson and A. Kim Ritchey.

Chairman of Steering Committee: Dr Wendell F. Rosse.

Statistical Coordinating Center: University of Illinois School of Public Health, Chicago; Dr Paul Levy, Dianne Gallagher, Adrienne Koranda, Zanet Fournory-Gill, and Emma Jones.

National Heart, Lung, and Blood Institute: Drs Marilyn Gaston, Joel Verter, and Clarice D. Reid.

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Transfusion and alloimmunization in sickle cell disease. The Cooperative Study of Sickle Cell Disease