Blood Transfusions and Immunophenotypic Alterations of Lymphocyte Subsets in Sickle Cell Anemia

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Transfusions purportedly induce dysfunction of cell-mediated immunity in sickle cell anemia (SCA). We studied hematologic and lymphocytic indices in 173 human immunodeficiency virus (HIV)-negative subjects with SCA and 131 black controls. Children aged 1 to 7 years with SCA had leucocyte counts and percentages of granulocytes, monocytes, natural killer cells, and T-cell markers (CD2, CD11b, CD4, CD28, CD4, CD29) that were significantly higher than those for control children. Percent total lymphocytes was decreased for this age group, but the total number of lymphocytes and T and B cell counts were similar to controls. Platelets were not increased. Adolescents (aged 8 to 17 years) and adults

Aquired functional asplenia in sickle cell anemia (SCA) is associated with inefficient phagocytosis of opsonized bacteria outside of the spleen. Whether other components of the immune system, including cellular immunity, are also compromised has been debated. The balance of the evidence is that cellular immunity is either not affected at all or at least is not clinically impaired. SCA patients, for example, do not have the incidence of opportunistic infections or severe forms of childhood viral diseases seen in immunocompromised persons. On the other hand, it has been suggested that the multiple transfusions necessary for many patients with congenital anemias may alter immune function by one or several mechanisms.

We report a prospective, observational, multicenter study of 173 human immunodeficiency virus (HIV)-negative SCA patients (104 transfused, 69 untransfused) and 131 black controls. The major purpose for our study in 1984 was to monitor for HIV-type 1 (HIV-1) among persons receiving repeated blood transfusions. When enrollment began in August 1985, only two persons with SCA in our cohort had HIV-1 infection. By that time, routine blood donor screening for anti–HIV-1 had already reduced this modest risk of transfusion acquisition of HIV-1 to an almost imperceptible level, and observations of SCA were discontinued in February 1989.

The broad scope of the Transfusion Safety Study, nonetheless, also included the definition of any changes in immunologic function attributable to blood transfusion. The data we collected gave us the opportunity to examine the hematologic indices and lymphocyte immunophenotypic distributions in SCA as a first step. We then gave particular attention to differences in immunophenotypic lymphocyte populations attributable to the administration of blood.

MATERIALS AND METHODS

The national multicenter Transfusion Safety Study had all protocols and questionnaires approved by institutional review boards at each participating site. Written consents for participation, including specific mention of anti–HIV-1 testing, were obtained from all subjects or their legal guardians.

Patients. The centers that participated in recruiting and observing patients with congenital anemias were located in New York, NY; Miami, FL; Detroit, MI; San Francisco, CA; and Los Angeles, CA. Recruitment, evaluation, and data collection for other categories of subjects are described elsewhere. For patients with congenital anemias, attention was focused on enrolling persons who required transfusion most frequently and those who almost never required blood components. As controls, we recruited the patients’ household members.

The total of 205 subjects had hemoglobin SS and characterized themselves as black. For the present study, observations were made when the patients were clinically well and crisis-free. Thirty-two patients have been excluded from the hematologic and immunologic analyses for the following conditions: HIV-1 infection (two patients), excessive numbers of nucleated erythrocytes (nRBCs; seven patients), age less than 12 months, malignancy, diabetes mellitus, pregnancy, and administration of immunosuppressants (including corticosteroids) and immunoglobulin preparations. For this SCA cohort of 173 subjects, we recruited 131 household members in generally good health who also identified themselves as black; they were not evaluated for sickle cell trait.

Blood transfusion history. Patients were categorized as transfused if review of their medical records at the clinic that they regularly attended showed no blood component therapy in the 2 years before study entry. If medical records were incomplete or not available, we reviewed blood bank records at study hospitals. For possible transfusions at other hospitals, we relied on the subject’s history.

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During follow-up observations every 6 months, the kind and amount of blood components were recorded for the interval since the prior visit. The coordinating center made specific inquiry as to whether transfusions were given for (1) prophylaxis to prevent medical complications (eg, cerebrovasculopathy and chronic lung disease) or (2) on demand (ie, transfusion to manage a limited clinical circumstance).

Virologic testing. Anti–HIV-1 status was tested at the coordinating center laboratory by enzyme-linked immunosorbent assay (ELIA) of both the initial and all serially collected sera during follow-up (Abbott Laboratories, North Chicago, IL). Confirmation was by Western immunoblot assay. During follow-up, a transfused person was not evaluated for anti–HIV-1 seroconversion unless there was at least one specimen tested ≥6 months later.11

Hematologic and lymphocyte immunophenotypic values. Each clinical center performed complete blood counts, usually by automated hemocytometers. If nRBCs are present in a blood specimen, these counters overestimate the total leukocyte count [white blood cell (WBC) count] and the percentage of lymphocytes. For subjects with a known congenital anemia, we performed a manual count and accordingly corrected the WBC count and the percentage of leukocytes.

Mononuclear cell subsets were estimated using a whole blood staining technique10 and two-color flow cytometry (Coulter EPICS-C, Hialeah, FL). All immunology laboratories used the same lots of monoclonal antibodies (Coulter Diagnostics, Hialeah, FL) and standardized protocols.13,14 The monoclonal antibodies to CD antigens were paired to monitor broadly the peripheral blood immune cells, including T cells, B cells, and natural killer (NK) cells, and to provide a detailed assessment of the CD4+ and CD8+ T cell subsets (Table 1).

Red blood cells were lysed with a modified ammonium chloride solution (Immunolyse, Coulter Diagnostics). However, nRBCs are variably ruptured by this procedure, so that no standard correction based on number of nRBCs per 100 WBCs could be used. Attempts to base a correction on anti-glycopherin to identify nRBCs were not successful.

In our population of SCA patients, 18 (10%) had ≥five nRBCs per 100 WBCs; the highest number was 49 nRBCs per 100 WBCs. Seven patients in our SCA population had ≥five nRBCs and a sum of lymphocyte subsets less than 85% of the lymphocyte count. We interpreted this cytogram to mean that the subsets were underestimated because of nRBCs in the lymphocyte gate. These seven SCA patients have been excluded from the analyses.

Statistical analyses. Values were statistically similar for males and females in this evaluation, and they have been analyzed together. Age-specific comparisons were made using three strata: age 1 to 7 years (n = 30), age 8 to 17 years (n = 65), and age ≥18 years (n = 78). Each patient was represented only once. The number of children was too small to permit further stratification.

To compare the values by age stratum, we used the Student-Newman-Keuls pairwise comparison to adjust for the significance level of multiple testing. There was homogeneity of age distributions within the 1- to 7- and 8- to 17-years strata by Wilcoxon rank sum testing. There was nonhomogeneity in age distribution among adults; adult SCA patients were 7.9 years younger than controls. Comparison of mean values for SCA and control adults with and without age adjustment showed only minor differences in the levels of statistical significance. The values we report for this stratum, therefore, are unadjusted.

The distributions in cell populations were normalized by a logarithmic or a square-root transformation15; only the distribution of percent CD4+ cells was normal without transformation. The age adjustments for each of the hematologic and mononuclear indices were made by fitting regression models to the normal values for each individual. For most variables, this procedure showed age as a linear function; in some instances, however, a quadratic equation was more appropriate. The age chosen for standardization was 18 years.

The two-tailed t test was applied to compare values in the same age group between the two populations. Exact testing was also used. All P values are two-tailed.

RESULTS

HIV-1 observations. At the time of entry into the study, only 2 (1.0%) of the 205 persons with SCA were infected with HIV-1. One was an untransfused, asymptomatic woman who died shortly after entry in an automobile accident. The second patient was a woman with diabetes mellitus and other conditions given 12 units of blood in 1984. She was first observed in 1986 and died in 1989.

During the course of follow-up observations, a total of 4,222 units of blood components were given to 146 of the subjects. There were no seroconversions in this group (280 person-years of observation) or in the group of SCA nontransfused patients and control subjects (383 person-years of observation).

Hematologic values. Age-stratified comparison of SCA patients with black controls showed significantly increased numbers of WBCs (P < .0001), granulocytes (P = .0001), and monocytes (P = .0001; Fig 1). These populations were increased by 1.8- to 2.7-fold above the controls. The granulocyte percentage of the WBCs in SCA patients and controls was the most elevated in the children (56.4% and 38.9%, respectively; P = .0001); for monocytes, the only significant difference was among the adults (5.6% and 4.5%, respectively; P = .02).

Platelet counts were similar among children with SCA (3.88 × 105 cells per microliter) and controls (3.60 × 105 cells per microliter; Fig 2). Among patients with SCA aged ≥8 years, platelet counts were higher, reaching mean ranges of 4.64 × 105 and 4.20 × 105 cells per microliter for adoles-

| Table 1. Peripheral Blood Mononuclear Cell Designations and Associated Functions |
|-----------------------------|-----------------------------|
| Phenotype | Associated Functions |
| CD14* | Monocytes |
| CD20* | All B cells |
| CD20*CD21* | Resting B cells |
| CD20*CD21* | Includes activated B cells |
| CD2* | Includes all T and NK cells |
| CD2*CD11b* | Includes a subset of NK and CD8+ cells associated with suppressor activity |
| CD2*CD26* | Includes T cells with enhanced proliferative response and memory |
| CD4* | Helper/inducer T cells |
| CD4*CD45RA* | Unprimed/naive T cells; includes suppressors for Ig production |
| CD4*CD45RA* | Includes primed T cells involved in recall response to antigens |
| CD4*CD29* | Primed T cells; includes helpers for Ig production |
| CD8* | Includes a subset of NK and suppressor/cytotoxic T cells |
| CD8*HLA-DR* | Activated suppressor/cytotoxic T cells |
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Fig 1. Mean (±95% confidence interval [CI]) hematologic values for three age groups of persons with SCA and black controls. The indices are total leukocyte count (□), granulocyte count (■), lymphocyte count (●), and monocyte count (□). The means are categorical variables; the connecting lines are for convenience in visualizing age trends.

Fig 2. Mean (±95% CI) platelet counts for three age groups of persons with SCA and black controls. The means are categorical variables; the connecting lines are for convenience in visualizing age trends.

Fig 3. Age group percentages of the absolute lymphocyte count in SCA patients and black controls attributed by flow cytometry to NK cells (CD56⁺; solid black), B cells (CD20⁺; hatched) and all T and NK cells (CD2⁻; open).

Monocytes defined by manual differential were uniformly increased in SCA over controls by 1.2- to 1.3-fold, but only the adult increase was statistically significant between SCA and controls (P = .02; Fig 1). Monocytes defined as CD14⁺ by flow cytometry demonstrated more of a monocytosis in SCA than the manual differential count. The counts for SCA patients aged 1 to 7 years, aged 8 to 17 years, and ≥18 years were 567, 805, and 836 cells/µL, respectively, compared with 329, 332, and 249 cells/µL among the controls (P < .0001).

For children, lymphocyte counts were not significantly different between SCA (4,412/µL) and controls (3,782/µL). Lymphocyte percentages among WBCs, however, were significantly (P < .0001) lower in SCA (36.1%) than in controls (55.9%). In adolescence (age 8 to 17 years) and adulthood (age ≥18 years), lymphocyte counts in SCA remained in essentially the same numeric range as in childhood (ie, greater than 4,200/µL), but their percentages among WBCs decreased to 34.0% and 32.7%, respectively. Controls in these two older age groups had age-related decreases in lymphocytes in respective counts (2,663/µL and 2,276/µL), as well as percentage (41.1% and 36.9%) decreases. Consequently, lymphocyte counts were significantly higher in adolescents and adults with SCA compared with controls (P = .0001).

**Immunophenotypic results.** NK cells (CD56⁺) in SCA were numerically increased over controls in all three age strata (P ≤ .0001; mean values for SCA patients were 509/µL, 415/µL, and 389/µL, respectively; for controls, 187/µL, 143/µL, and 182/µL, respectively. As a consequence, NK cells comprised a significantly higher percentage (P = .0001) of lymphocytes across all age strata (Fig 3).

B cells (CD20⁺) among SCA children were not significantly increased over controls, either numerically or as a percentage. Adolescent and adult SCA patients retained essentially the same mean counts as the children (494/µL, 557/µL, and 544/µL, respectively), while the average numbers among controls declined from the childhood level (544/µL, 328/µL, and 223/µL, respectively). Consequently, total B
cell counts were significantly higher in adolescents and adults with SCA compared with controls (Fig 3). The percentage of B cells became significantly higher in SCA adolescents and adults ($P < .0001$; Fig 3).

T cells (as estimated by CD27) had the same age-specific trend as B cells in SCA and controls. Children in both categories had similar counts in childhood (3,308/µL and 2,868/µL, respectively). SCA patients continued to maintain that level (greater than 3,000/µL) in adolescence and adulthood, while controls had decreasing numbers (2,077/µL and 1,873/µL, respectively). This difference between SCA patients and controls was significant for the two older age groups ($P < .0001$ for both strata). As a percentage of lymphocytes (Fig 3), only the high value (80.7%) among adult controls was statistically significant ($P < .05$).

Table 2 compares mean counts for subsets of B and T cells. Resting B cell counts (CD20+CD21-) were the same in children and became lower in both SCA patients and controls with adolescence and adulthood. The decline, however, was much less for SCA patients than for controls. Activated B cell counts (CD20+CD21+) were also similar in SCA and control children, but were higher in anemic adolescents and adults.

In SCA patients, T cells expressing CD11b and CD26 were much more numerous than in controls at all ages. Total numbers of CD4+ cells as well as CD8+ cells and subsets had patterns that paralleled the lymphocyte counts. CD4+, CD8+, and CD8+HLA-DR+ cells showed no downward or upward trend with SCA across age groups, compared with steadily lower counts for adolescent and adult controls.

To delineate the many changes in percentages, counts, and their proportions, we have summarized the differences between SCA patients and controls in each age group by indicating the value for the former to that of the latter as a ratio (Table 3). Because changes were very numerous in the percentage and/or number of each major subpopulation, the respective subsets were expressed as proportions of the "parent" ontologic lineage. For example, CD20+CD21+ and CD20+CD21- are expressed as ratios of the percent and number of CD20+ cells.

Among children with SCA, monocytes and NK cells were increased both as percentages (1.5- and 2.2-fold, respec-
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Table 3. Means of Age-Specific Ratios of Percentages and Counts in SCA Patients Compared With Black Controls

<table>
<thead>
<tr>
<th>Cell Population</th>
<th>Ratio of Mean Percentages Comparing SCA Patients With Controls</th>
<th>Ratio of Mean Counts Comparing SCA Patients With Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ratio Denominator*</td>
<td>Age 1-7 yr</td>
</tr>
<tr>
<td>Monocytes (CD14+)</td>
<td>PBMC</td>
<td>1.5±</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>WBC</td>
<td>0.6±</td>
</tr>
<tr>
<td>CD56*</td>
<td>ALC</td>
<td>2.2±</td>
</tr>
<tr>
<td>CD20*</td>
<td>ALC</td>
<td>NS</td>
</tr>
<tr>
<td>CD20<em>CD21</em></td>
<td>CD20*</td>
<td>NS</td>
</tr>
<tr>
<td>CD20<em>CD21</em></td>
<td>CD20*</td>
<td>NS</td>
</tr>
<tr>
<td>CD2*</td>
<td>ALC</td>
<td>NS</td>
</tr>
<tr>
<td>CD2CD11b*</td>
<td>CD2*</td>
<td>1.7±</td>
</tr>
<tr>
<td>CD2CD26*</td>
<td>CD2*</td>
<td>1.6±</td>
</tr>
<tr>
<td>CD4*</td>
<td>CD2*</td>
<td>NS</td>
</tr>
<tr>
<td>CD4<em>CD45RA</em></td>
<td>CD4*</td>
<td>NS</td>
</tr>
<tr>
<td>CD4<em>CD45RA</em></td>
<td>CD4*</td>
<td>NS</td>
</tr>
<tr>
<td>CD4<em>CD29</em></td>
<td>CD4*</td>
<td>1.3±</td>
</tr>
<tr>
<td>CD8*</td>
<td>CD2*</td>
<td>NS</td>
</tr>
<tr>
<td>CD8<em>HLA-DR</em></td>
<td>CD8*</td>
<td>2.1±</td>
</tr>
</tbody>
</table>

Abbreviations: PBMC, peripheral blood mononuclear cells; WBC, leukocyte count; ALC, lymphocyte count.
* The control denominator cell population for the ratio of the subpopulation in the first column.
† .005 < two-sided P < .05 by pairwise t test.
‡ Two-sided P = .05 by pairwise t test.

Hematologic indices (Table 4) varied among the three transfusion categories. Overall, the distributions of leukocyte and granulocyte counts were statistically very significantly higher with regular transfusion (P ≤ .001). Platelet and lymphocyte counts were not significantly different; monocyte counts overall were at borderline significance (P = .03), but pairwise comparisons were not significant.

For the prophylactic transfusion category, the leukocyte and granulocyte counts were both significantly higher by pairwise comparisons than for either of the other two categories. Comparisons of the peripheral blood mononuclear cell subpopulations showed no statistically significant differences among the three transfusion categories.

DISCUSSION

There are several plausible explanations for increased counts in the peripheral blood of patients with SCA. Altered trafficking in asplenic subjects is probably a factor in thrombocytosis, monocytopsia, and lymphocytosis.16 The broad-based increases in T, B, and NK cells may also reflect lymphocyte activation caused by infections and tissue damage and/or increased bone marrow production. Transient lymphocytosis has been reported during sickle cell crisis, but the CD4:CD8 ratio is variably affected.18,19

In SCA, the number and proportion of CD2*CD11b*, CD2*CD26*, and CD4*CD29* cells is increased in childhood (Table 3). This may reflect activation of primed T cells as the frequency of infection increases with progressive hyposplenism. In adolescence and adulthood, the number of activated cells remains high, although their proportion is no longer significantly increased. In adults with SCA, we also observed an increase in both number and proportion of activated B cells. We suggest that this B-cell activation reflects immune stimulation secondary to tissue damage.

tively) and counts (1.7- and 2.7-fold, respectively) compared with controls. Lymphocytes were decreased (0.6) as a percentage of WBCs, but their counts were similar. Children had no T-cell changes other than in the percentage and number expressing the CD11b and CD26 markers, and in an increased proportion and number of primed cells (CD4*CD29*) among CD4+ cells.

Among adolescents and adults with SCA, the size of all subpopulations and their subsets were significantly increased. For proportionate changes, among SCA adults the percentages of B cells (CD20+) and activated B cells (CD20*CD21+) significantly increased (1.3- and 1.1-fold); the proportion of resting B cells declined (0.8). The percentage of T cells in adolescents and adults was slightly but significantly lower (0.9). The percentages of some activation markers were variably increased.

Effect of transfusion on lymphocyte subsets. During the course of observation, 69 (40%) patients were given prophylactic blood transfusions, 42 (24%) patients received occasional transfusions as needed, and 62 (36%) patients had been transfused in the 2 years before study entry. The age distributions in the three transfusion groups were homogeneous; the medians were 14, 22, and 15 years, respectively (Kruskal-Wallis test, P = .002). The percentages of children, adolescents, and adults receiving prophylactic transfusions were 30%, 54%, and 30%, respectively. Accordingly, values have been adjusted to a standard age of 18 years for the comparisons of the transfusion effect. The patients on prophylactic transfusions had a mean intensity of 12.6 units per 6 months (mean duration of follow up, 33 months; mean number of transfusions, 73 units). Those treated on demand had a mean intensity of 1.4 units per 6 months (mean duration of follow up, 27 months; mean number of transfusions, 6.4 units).
Monocytes and NK cells in SCA were increased compared with controls in both percent and number (Table 3). A threefold increase in the percent and number of monocytes has been previously reported in SCA. It has been hypothesized that this monocytosis arises as an increased tissue phagocytic capacity in response to hemolysis or tissue damage.

It is also possible that the monocytosis and increase in NK cells in SCA augments host defense. Both monocytes and NK cells participate in antibody-dependent cellular cytotoxicity mechanisms that provide important and effective responses to viral infections.

Neither the extent nor frequency of transfusion altered the generalized lymphocytosis seen in SCA patients. Grady et al made a similar observation in splenectomized thalassemic individuals. We observed no transfusion-associated effect on NK cell numbers or CD4:CD8 ratios. These findings are in contrast to those of Escalona et al and others who reported in SCA patients a decrease in CD4+ CD8+ ratios attributed to increased CD8+ cells. We observed an increase in CD8+ cells balanced by an equal increase in CD4+ cells. Wang et al reported that CD4+ CD8+ ratios were unaffected by the numbers of units transfused in SCA patients undergoing chronic transfusions. Similarly, Hassett et al found no significant effect on CD4+ or CD56+ cells in patients with various clotting factor deficiencies receiving factor therapy.

The extent of transfusion therapy is closely linked to disease progression and severity. Rather than being a transfusion effect, the higher number of WBCs, granulocytes, and monocytes in childhood patients receiving prophylactic transfusions may arise from progression of severe ischemic microvasculopathy beginning at an early age. We observed a similar increase in WBCs, granulocytes, and monocytes in untransfused children with SCA compared with controls. The underlying disease process in SCA, therefore, may cause increased WBC and granulocyte counts. Demeter et al observed monocytosis and lymphocytosis but no granulocytosis in patients after posttraumatic splenectomy.

In this report we present evidence that a broad-based leukocytosis is characteristic in SCA at all ages, regardless of transfusion history. Granulocytes and monocytes participate in the increase relatively more than lymphocytes. All T- and B-cell subsets participate in the lymphocytosis in SCA, although primed, activated T cells are relatively prominent in childhood SCA and activated B cells in adult SCA. Our findings do not distinguish the relative contributions to increased cell numbers in SCA of increased production, altered trafficking, immune stimulation, and the infections and tissue damage present. Our findings suggest that transfusions in SCA do not significantly alter immune competence, as measured by the peripheral blood phenotypic profile.

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APPENDIX

The following persons either have responsibility at present for the conduct of the Transfusion Safety Study Group or contributed particularly to the present report: J.W. Mosley, J. Buckley, M. Hanis, C.K. Kasper, E.A. Operskalski, M.C. Pike, and B. Operskalski (University of California, Los Angeles, CA); J.M. Lusher, J. Kaplan, and S. Sarnaik (Wayne State University, Detroit, MI); E.R. Schiff, M.A. Fletcher, C.H.
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