ACQUIRED IMMUNODEFICIENCY syndrome (AIDS), a frequently lethal disorder that has occurred most frequently in homosexual males and intravenous drug abusers, has also been detected in a small number of hemophiliacs treated with factor (F) VIII or FIX concentrates and in some recipients of blood transfusions, prompting concern that AIDS may be due to an infectious agent transmissible through blood products. This concern has been heightened by the observation that some of the immunologic abnormalities characteristic of AIDS, including diminished ratio of helper to suppressor lymphocytes and low NK cell activity, have been found, albeit to a lesser degree, in many otherwise healthy hemophiliacs who receive repeated injections of FVIII or FIX concentrates derived from multiple blood donors. Such changes could reflect a high frequency of subclinical infection with the putative AIDS agent(s) in hemophiliacs who receive clotting factor concentrates. Alternatively, they could represent normal immunologic responses to repeated antigenic stimulation by such ubiquitous blood-borne viruses as Epstein-Barr virus (EBV), cytomegalovirus (CMV), and hepatitis B virus (HBV), or by alloantigens. In support of this latter possibility, we now report that the same pattern of immunologic alterations that occurs in hemophiliacs receiving clotting factor concentrates also occurs in subjects receiving repeated blood transfusions.

RESULTS
Hemophiliacs and transfused SCA patients both had low ratios of helper to suppressor (T4/T8) lymphocytes and low NK activity. However, the alterations in hemophiliacs were observed in the absence of a history of transfusions, suggesting that these changes may be due to the presence of an infectious agent.

MATERIALS AND METHODS

Subjects
Blood was obtained from four groups of subjects: (A) 30 subjects with sickle cell anemia (SCA) and previous central nervous system (CNS) infarctions (aged 4–30 years), who were receiving monthly partial exchange transfusion to prevent recurrent episodes of CNS infarction ("transfused-SCA"); (B) 30 nontransfused subjects with SCA (aged 5–32 years), who had not received a transfusion for at least a year preceding the study; (C) 87 hemophiliacs (aged 2–61 years), and (D) 30 normal controls (aged 15–56 years). Blood specimens were obtained from the transfused SCA subjects just prior to monthly partial exchange transfusion in which 5 mL/kg blood was removed prior to transfusing 10 mL/kg of sedimented washed erythrocytes. Blood specimens were obtained from the nontransfused SCA subjects and hemophiliacs at the time of routine follow-up clinic visit. None of the subjects had evidence of infection at the time of sampling.

Specimen Preparation
Mononuclear cells were obtained from heparinized venous blood by Ficoll-Hypaque separation. This was performed within two hours of venipuncture to avoid any storage-induced artifacts in enumerating lymphocyte subpopulations.

Lymphocyte Markers
Mononuclear cell suspensions were tested by indirect immunofluorescence for cells with T4 (helper) or T8 (suppressor) phenotype, as determined by reactivity with OKT monoclonal antisera (Ortho Diagnostic Systems, Raritan, NJ).

NK Activity
The NK activity of each subject was determined as previously described by measuring spontaneous killing of tumor cell line K562 in a 4-hour 51Cr-release assay. The cytotoxicity data so generated were analyzed by an exponential fit equation to yield the number of lytic units/K562 cells, where 1 lytic unit is the number of effector cells required to cause 20% specific lysis of 5 × 10⁵ labeled target cells.

REFERENCES
Hemophiliacs and transfused SCA patients both had low ratios of helper to suppressor (T4/T8) lymphocytes and low NK activity. However, the changes in hemophiliacs were observed in the absence of a history of transfusions, suggesting that these changes may be due to the presence of an infectious agent.
low NK activity compared to normal controls (P < .0005) (Fig 1). By contrast, nontransfused SCA patients had values that did not differ significantly from normal. This, together with the fact that nontransfused and transfused SCA patients had a similar age distribution, indicates that the diminished T4/T8 ratios and NK activity seen in the latter group were primarily related to multiple transfusions and were not age-related or specific for sickle cell anemia.

The absolute and relative number of total lymphocytes and helper and suppressor lymphocytes are given in Table 1. It would appear that the lowering of the T4/T8 ratio in transfused SCA subjects compared to nontransfused SCA subjects was primarily due to a relative increase in percent T8+ cells, as the percent T4+ cells in the transfused group was actually higher than the percent T4+ cells in the nontransfused group.

DISCUSSION

The results indicate that the immunologic pattern of diminished helper suppressor lymphocyte ratios and low NK activity, which is characteristic of AIDS, occurs to a lesser degree, not only in hemophiliacs who are repeatedly infused with clotting factor, but also in subjects who receive repeated blood transfusions.

These immunologic alterations appear to be transfusion-associated and not specific for sickle cell disease, because they do not occur in nontransfused SCA subjects. Moreover, previously reported preliminary findings indicate that diminished T4/T8 ratios occur not only in hypertransfused patients with SCA, as described here, but also in hypertransfused subjects with thalassemia.

It can be argued that transfusion-acquired infection with such common blood-borne viruses as EBV, CMV, and HBV is the most likely cause of diminished helper suppressor ratios in transfused subjects. Like hypertransfused patients and hemophiliacs, subjects acutely infected with EBV and CMV usually have low helper suppressor ratios, accompanied by increases in relative and absolute numbers of suppressor cells, with only a relative reduction in helper cells. However, transfusion-acquired infection with these viruses does not readily account for lowered NK activity, since NK activity during such infections has been observed to be normal or increased.

An alternative mechanism, which, by itself, could account for the association between repeated blood transfusion and lowering of both helper suppressor ratios and NK activity, is that both changes may be part of the normal immune response to chronic alloantigenic stimulation. Thus, bone marrow transplant recipients, in whom donor-derived lymphoid cells are chronically exposed to the recipient's foreign histocompatibility antigens, have prolonged reduction of helper suppressor lymphocyte ratios, and 20% have low to absent NK activity even as long as 1 year following transplant. In addition, prolonged depression of NK activity has been observed in experimentally induced chronic graft-versus-host disease, another situation characterized by chronic alloantigenic stimulation.

Based on such findings, Shearer has proposed that repeated exposure to foreign histocompatibility antigens may be a major factor in the pathogenesis of disease patients, and normal controls.

Fig 1. Helper suppressor (T4/T8) lymphocyte ratios and NK activity in hemophiliacs, transfused and nontransfused sickle cell disease patients, and normal controls.

Table 1. Absolute and Relative Numbers of Total Lymphocytes and T4+ and T8+ Lymphocytes (Mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Controls (N = 30)</th>
<th>Transfused SCD (N = 30)</th>
<th>Nontransfused SCD (N = 39)</th>
<th>Hemophiliacs (N = 89)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes/μL</td>
<td>2,113 ± 262</td>
<td>5,300 ± 392§</td>
<td>4,331 ± 275§</td>
<td>2,752 ± 262*</td>
</tr>
<tr>
<td>Percent</td>
<td>37 ± 5</td>
<td>32 ± 2</td>
<td>37 ± 3</td>
<td>44 ± 2</td>
</tr>
<tr>
<td>OKT4/μL</td>
<td>1,096 ± 118</td>
<td>1,991 ± 165§</td>
<td>1,291 ± 77</td>
<td>1,360 ± 104</td>
</tr>
<tr>
<td>Percent</td>
<td>49 ± 2</td>
<td>33 ± 2§</td>
<td>27 ± 1§</td>
<td>42 ± 1§</td>
</tr>
<tr>
<td>OKT8/μL</td>
<td>604 ± 67</td>
<td>1,386 ± 118§</td>
<td>836 ± 82*</td>
<td>1,176 ± 74†</td>
</tr>
<tr>
<td>Percent</td>
<td>28 ± 1</td>
<td>24 ± 1†</td>
<td>17 ± 1§</td>
<td>38 ± 1§</td>
</tr>
</tbody>
</table>

Level of significance of difference compared to controls:
*P < .05.
†P < .025.
‡P < .005.
§P < .0005.
AIDS.\textsuperscript{12} If so, the present findings indicate that most subjects respond to such exposure with a modest decrease in helper/suppressor ratio and NK activity. By contrast, rare individuals may be prone to developing a markedly exaggerated and irreversible form of this response, which renders them severely immunodeficient. Further investigation of the normal immunologic response to transfusion could lead to methods for identifying subjects who may be uniquely susceptible to AIDS.

REFERENCES

Diminished helper/suppressor lymphocyte ratios and natural killer activity in recipients of repeated blood transfusions

J Kaplan, S Sarnaik, J Gitlin and J Lusher