Immune Status of Blood Product Recipients

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• **Persons with hemophilia are at risk of the acquired immunodeficiency syndrome (AIDS), and clinically asymptomatic hemophiliacs have shown a high incidence of AIDS-like immune abnormalities, facts leading to speculation that many hemophiliacs have been exposed to the AIDS agent through their blood products. We therefore evaluated the immune status of three groups of blood product recipients without AIDS in New York City, including 47 persons with hemophilia A receiving factor VIII concentrate, 50 persons with homozygous β-thalassemia, and 27 persons with sickle cell anemia receiving frozen-packed RBCs and 20 healthy persons who had not received a transfusion. Hemophiliac participants had significantly lower lymphocyte counts (median, 1,826/cu mm) than did the thalassemic (6,110/cu mm) or anemic (4,443/cu mm) participants, had lower numbers of T-helper lymphocytes (median, 533 cells/cu mm *v* 1,733 cells/cu mm and 1,554 cells/cu mm), and had a lower T-helper/suppressor ratio (median, 0.8 *v* 1.8 and 2.1). These differences remained after adjustment for age and sex. Thus, AIDS-like immune abnormalities were found in patients receiving factor concentrate, but not in those receiving RBCs. These defects could be due to both an immunosuppressive effect of the lyophilized factor itself and to contact with the AIDS agent.**

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PERSONS with hemophilia are known to be at heightened risk for the acquired immunodeficiency syndrome (AIDS).[1] Nine of the patients with hemophilia-associated AIDS whose cases were reported to the Centers for Disease Control (CDC) (Atlanta) had no other risk factors for AIDS and had received no blood products other than factor concentrate. Additionally, recent studies have shown that clinically normal persons with hemophilia have high rates of immune abnormalities, including decreased numbers and/or percentage of T-helper/inducer (T_H) lymphocytes,[2-10] increased numbers and/or percentage of T-suppressor/cytotoxic (T_S) lymphocytes,[2-5,10-12] decreased T_H/T_S ratio,[2-10,13-15] and increases in one or more immunoglobulin classes.[2-4,5,8,12,13] The relationship between these findings and AIDS is unclear, but one study of temporal trends in lymphocyte counts for hemophiliacs in North Carolina suggests that these counts have decreased since the onset of the AIDS epidemic in the United States.[16] It has thus been suggested that persons with hemophilia may contact the AIDS agent via the blood products they receive as therapy for their hematologic disorder.[1,13]

Cases of transfusion-associated AIDS in nonhemophiliacs support the probability that AIDS is caused by an agent transmissible through blood products.[17] Some studies have shown a correlation between use of factor VIII concentrate and immune abnormalities in clinically asymptomatic patients with hemophilia.[3-6,10,11,13,15] Two studies found a correlation with factor VIII concentrate yearly dosage[10,15] and two did not.[5,8] Determining the role of factor is complicated by the fact that factor is sometimes not the only blood product received by hemophiliac patients. Furthermore, immune abnormalities have been found in at least four hemophiliac populations with little or no contact with US-produced, and thus presumably high-AIDS-risk, factor concentrate.[18-22] Thus, it has been suggested that factor concentrate and/or other blood products may themselves be

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immunosuppressive.[7,10] To help clarify the relationship between immune abnormalities in asymptomatic hemophiliac patients and these patients' use of various blood products, we compared the immune findings for patients with hemophilia who were receiving factor VIII concentrate therapy with two patient groups who were receiving long-term cellular blood product therapy, ie, patients with thalassemia or sickle cell anemia, and with healthy persons who had not received a transfusion.

PARTICIPANTS AND METHODS

Participants were enrolled in 1983. Forty-seven participants with hemophilia A (Hem A) who had received 100,000 units or more of factor VIII concentrate in the preceding 12 years were enrolled from two treatment centers in New York City. These centers follow up approximately 40% of the persons being treated for hemophilia in that city. Fifty participants with homozygous β-thalassemia (Thal) were enrolled from a center following up 67% of the thalassemic patients receiving routine transfusion therapy in New York City. These recipients had received 12 to 24 units of frozen-packed RBCs (FPRBCs) per year in the previous three years to maintain a hemoglobin level of 11 g/dL or higher. Twenty-six patients with homozygous sickle cell anemia (SCA) and one patient with sickle thalassemia, who was included in the SCA group, were enrolled from three centers in New York City that use routine transfusion therapy. These persons received 6 units or more of FPRBCs per year during the previous three years. All patients were enrolled during routine clinic visits, not during episodes of acute exacerbation of disease. Blood specimens were also obtained from 20 healthy New York City medical care personnel who volunteered to be controls for laboratory testing.

Three years of information about factor use was obtainable for 42 of the patients with Hem A, who had received a median of 396,741 units (range, 16,470 to 1,444,260 units) of factor VIII concentrate during the three years preceding their laboratory studies. Only five had received any cellular blood products; these were for isolated bleeding incidents during the previous five years. Three years' information of blood product use was obtainable for 48 of the patients with Thal, who had received a median of 106.5 units of FPRBCs (range, 10 to 170 units) during the previous three years. Four of these patients with Thal had received other blood products at isolated points in time. Three years' information of blood product use was obtainable for 23 of the patients with SCA, who had received a median of 41 units of FPRBCs (range, 11 to 142 units) during the previous three years. One had also received plasma.

Immunologic testing using standard techniques was performed at the CDC.[23,24] Lymphocyte subpopulations were quantitated by indirect immunofluorescence on a fluorescence-activated cell sorter (Becton-Dickinson, Sunnyvale, Calif) using commercially available monoclonal antibodies (OKT3 for T cells, OKT4 for T_H cells, and OKT8 for T_S cells) (Ortho Diagnostics, Raritan, NJ) and a fluorescein-conjugated anti-mouse immunoglobulin (CDC).[24] Immunoglobulins G, A, and M were quantitated by nephelometry (Baker Chemicals, Allentown, Pa).

The immunologic results from these tests were not normally distributed for our study groups. Immunologic results were compared between groups using the Wilcoxon rank-sum test.[25] Spearman's rank correlation coefficient (r_s) was used to measure the strength of association between immunologic findings and the following variables[25]: time since splenectomy for Thal, age, and blood product use. To determine if a correlation was due to a common association with age or if the relation was a direct one, partial correlation coefficients were calculated to eliminate the effect of age. The partial correlation coefficient "$r_{1,2}$" here refers to the correlation, computed on ranks, between variables 1 and 2, with variable 3 held constant. Adjustments for age and sex differences in the comparison of immunologic results between groups were made using the analysis of covariance (ANCOVA), following rank transformation of the data.[26,27] Significance level for all statistical analyses was .05.

RESULTS

Differences in sexual and racial distributions between participant groups were unavoidable, given the three underlying diagnoses, and are outlined in Table 1. The median ages of Thal and SCA groups were significantly less than that of the Hem A group; this was probably due to the shorter life expectancy of persons with these diagnoses. All the control participants were male; ages ranged from 27 to 50 years, with a median of 30 years. Only one participant who had received a transfusion had a history of known risk factors for AIDS; this hemophiliac patient had a history of intravenous (IV) drug abuse, had not used IV drugs since January 1981, and was receiving methadone maintenance at the time of this study. A number of participants in all groups had lived in or traveled to Caribbean areas (33%, Hem A; 25%, SCA; and 16%, Thal). Having traveled or lived in the Caribbean or not was not correlated with any of the described immunologic findings.

Lymphocyte typing was done for each participant group (Table 2). Complete testing could not be done for all individuals. The Hem A group was the only one with a relatively low number of lymphocytes ($P=.025$ v controls), while the Thal and SCA groups had elevated numbers of lymphocytes ($P<.001$ for each, compared with controls). We reviewed random lymphocyte counts from between 1974 and 1980 for 23 participants with Hem A, 45 with Thal, and 21 with SCA. This review indicated that median and range of recent lymphocyte counts obtained for this study did not represent an appreciable decline from those obtained previously, ie, a random count obtained for each patient showed a median lymphocyte count of 1,885/cu mm (range, 960 to 4,095/cu mm) for patients with Hem A, a median of 5,148/cu mm (range, 576 to 21,676/cu mm) for patients with Thal, and a median of 4,305/cu mm (range, 1,030 to 8,840/cu mm) for patients with SCA. Temporal comparisons made on an individual basis also showed no regular pattern.

Hemophilia A was the only group with a relatively low number of T_H lymphocytes ($P<.0001$ v controls), while participants with Thal and SCA had increased numbers of T_H lymphocytes ($P=.001$ for Thal and $P=.018$* for SCA, compared with controls). All patient groups had an elevated number of T_S lymphocytes compared with controls, although the difference from controls was significant for Thal only ($P=.0005$* v controls). The T_H/T_S ratio was lower for Hem A (median, 0.85) than for Thal (median, 1.83; $P<.0001$), SCA (median, 2.08; $P<.0001$), or controls (median, 1.82; $P<.0001$) (Figure). The T_H/T_S ratio for men with SCA was not significantly elevated compared with that of

*The P value was obtained using ANCOVA to adjust for differences in age and/or sexual distributions between described groups. Unmarked P values indicate ANCOVA was not needed.

Table 1.—Characteristics of Participant Groups

Characteristic	Diagnosis: Hemophilia (n=47), %	Thalassemia (n=50), %	Sickle Cell Anemia (n=27), %	Control (n=20), %
Sex				
M	100	52	44	100
F	0	48	56	0
Total	100	100	100	100
Race				
W	87	100	0	U*
B	2	0	93	U
Other	11	0	7	U
Total	100	100	100	U
Age, yr				
Median	27	18	19	30
Range	13-52	9-36	7-57	27-50

*U indicates unknown.

Table 2.—Lymphocyte Populations, by Participant Group

Lymphocyte Population	Hemophilia (n=47)	Thalassemia (n=44)	Sickle Cell Anemia (n=22)	Control (n=20)
Total lymphocyte count, cells/cu mm				
Median	1,826	6,110	4,443	2,218
Range	1,066-4,277	1,736-17,298	1,054-8,398	1,425-4,017
T-helper count, cells/cu mm				
Median	533	1,733	1,554	976
Range	170-1,454	353-4,595	126-5,220	439-2,370
T-suppressor count, cells/cu mm				
Median	691	962	742	566
Range	261-1,754	106-2,768	53-1,436	326-1,077

the control group.

Patients in all three patient groups had similar IgG levels but showed significant elevations compared with the control group ($P<.0001$ for Hem A, $P<.0001$* for Thal and SCA) (Table 3). Elevations in IgA levels compared with the control group were statistically significant for Thal ($P<.0001$*), SCA ($P=.040$*), and Hem A ($P=.042$*). Differences in IgA levels between patient groups were significant only for Hem A *v* Thal ($P<.0001$*). Only Hem A had an elevation in IgM levels ($P=.001$* *v* controls, $P<.01$* *v* Thal, and $P<.01$* *v* SCA). There was no significant difference in IgM levels for Thal and SCA.

Forty-four participants with Thal had undergone splenectomy before this study; six had not. Time since splenectomy ranged from ten months to 31.3 years (median, 7.4 years). The numbers of lymphocytes, T_H and T_S, had no correlation with time since splenectomy. The correlation between the T_H/T_S ratio and time since splenectomy was of only borderline significance ($r_s=.29$, $P=.055$). There was no relationship between immunoglobulin levels and time since splenectomy (IgG, $r_{123}=.11$; IgA, $r_{123}=.21$, not significant; IgM $r_{123}=.16$; not significant). (Time since splenectomy was highly correlated with age [$r_s=.80$, $P<.0001$] and seemed to be correlated with immunoglobulin levels. Partial correlation coefficients were therefore calculated, removing the effect of the participants' ages.)

The quantity of factor VIII used by the hemophiliac participants for the entire three-year period or for individual years did not correlate with any cellular immune results or with immunoglobulin levels ($-.20\leq r_s\leq .18$ for each). (Usage in 1982 correlated with age [$r_s=-.33$, $P<.05$] and seemed to correlate with IgA levels [$r_s=-.31$, $P<.05$]; these relationships were not seen in the other two years or the entire three-year period. However, after adjustment for age, there was no association between usage and IgA levels for 1982 [$r_{123}=.24$, not significant].)

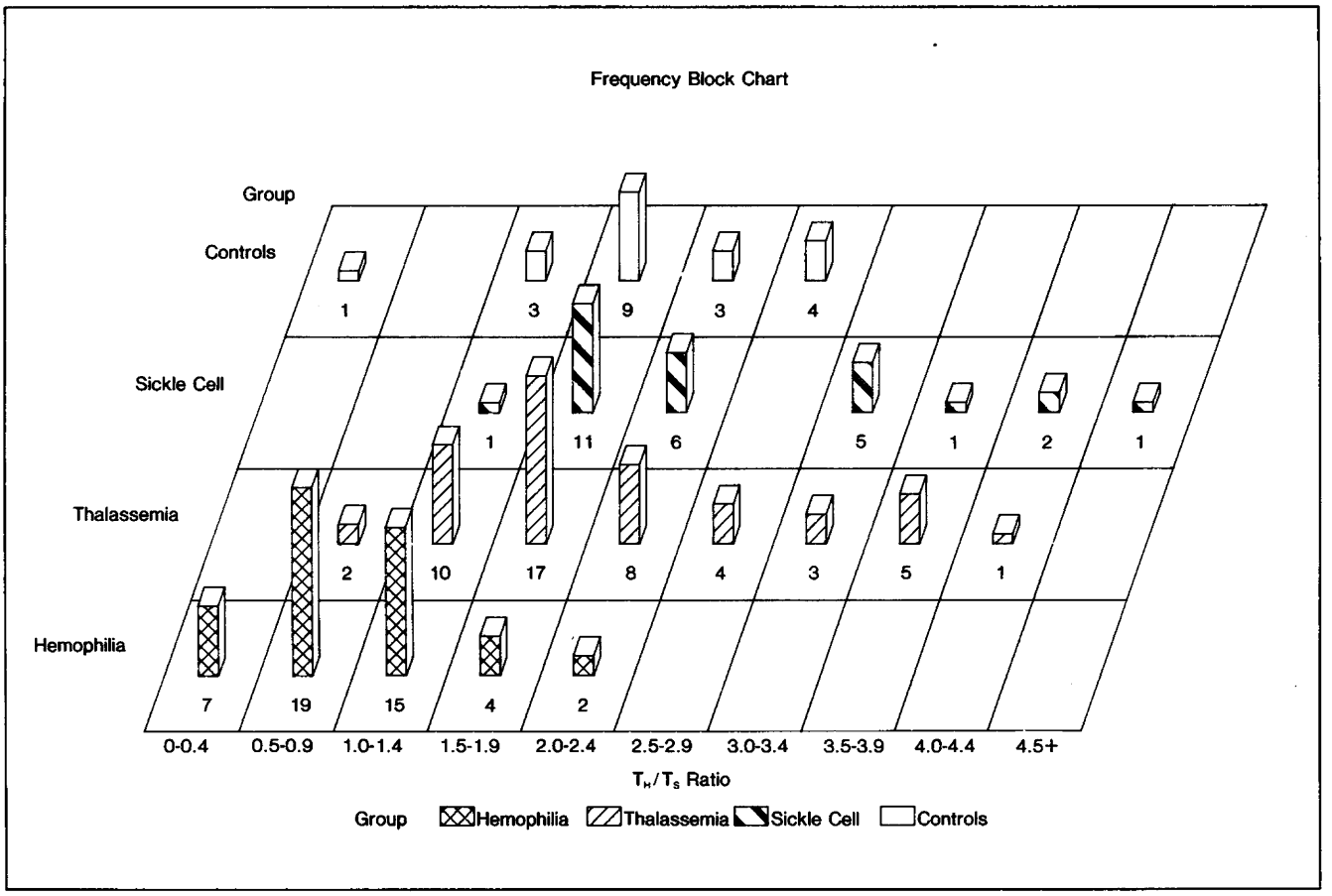
For Thal, the quantity of blood products used correlated with age for each year, and for the entire three-year period ($r_s=.47$, $P<.001$). Usage by Thal and SCA groups did not correlate with any cellular or humoral immune findings. (Usage by participant with Thal seemed to correlate with IgA [$r_s=.32$, $P<.05$] and IgM [$r_s=.39$, $P<.01$] levels, but these associations disappeared when partial correlation coefficients were calculated to control for age [$r_{123}=.07$, not significant for IgA and $r_{123}=.24$, not significant for IgM].)

COMMENT

As of Aug 10, 1984, forty-five cases of AIDS have been reported in an estimated US hemophiliac population of 14,467 factor VIII- or factor IX-deficient patients.[28] This represents a much higher risk of AIDS than that for the general US population[1] or for other blood product recipients. (In 1983, thirteen cases of AIDS were reported in persons with hemophilia [13/14,467, or one case per 1,113 hemophiliacs], compared with 28 cases of AIDS reported in an estimated 3 million persons receiving transfusions that year [one case per 100,000 transfusion recipients].[31]) At least nine of these persons reportedly received no blood products other than factor concentrate and had no risk factors other than hemophilia for AIDS.

Furthermore, during the period of the US AIDS epidemic (approximately 1980 to the present), many asymptomatic hemophiliacs have been found to have immune defects similar to those found in patients with AIDS[29] or with AIDS-associated conditions.[30] These defects include lymphopenia; a reduction in number or percentage of T_H lymphocytes; an increase in number or percentage of T_S lymphocytes; and polyclonal hypergammaglobulinemia, most often of the IgM class.[2-15] These data strongly suggest that factor concentrate can potentially transmit the AIDS agent.

However, other data strongly suggest that factor itself may be in some way immunosuppressive, perhaps by providing the patient with frequent and large IV antigenic burdens[7,10,32] or



Frequency distribution of T-helper/suppressor (T_H/T_S) ratios for participant groups (n=47 for hemophilia, n=50 for thalassemia, n=27 for sickle cell anemia, and n=20 for controls).

Table 3.—Immunoglobulins, by Participant Group

Immunoglobulins	Hemophilia (n=45)	Thalassemia (n=49)	Sickle Cell Anemia (n=27)	Control (n=20)
IgG, mg/dL				
Median	1,620	1,540	1,590	930
Range	1,070-3,580	565-3,820	847-3,470	730-1,450
IgA, mg/dL				
Median	192	280	238	157
Range	69-778	75-1,140	38-470	59-266
IgM, mg/dL				
Median	188	112	117	125
Range	75-795	20-433	48-251	57-351

by its biologic nature secondary to fractionation methods.[15] Probably the strongest evidence in favor of factor being directly associated with immune abnormalities, independent of possible AIDS transmission, is the abnormalities found in foreign hemophiliac populations receiving factor, little or none of which was derived from donations made by AIDS-prevalent populations.[18-22]

Red blood cell transfusions have been reported to be associated with allograft survival or laboratory immune abnormalities, but the changes described do not include those most frequently noted in AIDS.[32-35] Furthermore, no persons with transfusion-associated AIDS in the CDC-recorded cases had been receiving long-term transfusion therapy and, thus, these persons do not seem to be at heightened risk of AIDS. We therefore compared three types of patients receiving long-term blood product therapy with each other and with control (not receiving a transfusion) volunteers to examine whether immunologic findings differed for persons receiving factor and cellular blood products. The cellular blood products were derived from donations made preponderantly in New York City, a city with a high incidence of AIDS, and thus presumably were at some risk of AIDS contamination. Factor concentrates were produced from blood donations made in a broader geographic distribution.[1]

None of the blood recipient groups were immunologically like the control group of participants who had not received a transfusion. Patients with Thal had increased lymphocyte counts, increases of both T_H and T_S lymphocyte populations, a normal T_H/T_S ratio, and hypergammaglobulinemia involving the IgG and IgA subclasses. None of these findings showed any correlation with time since splenectomy, once age was taken into account. Patients with SCA, like the patients with Thal, had increased lymphocyte counts and increased numbers of T_H lymphocytes. Unlike the thalassemic patients, pa-

tients with SCA resembled the control group in regard to number of T_S lymphocytes. Immunoglobulin patterns for this group were similar to those of the patients with Thal, with elevated levels of IgA and IgG and normal levels of IgM. Our findings for hemophiliac patients differed significantly from those of the other two blood product recipient groups and from controls. Lymphocytes were reduced in number; T_H cells were reduced, and T_S cells were increased in number and proportion compared with the control group. Hemophiliac patients were also the only group with elevations of all three immunoglobulin classes, with IgA levels being significantly lower and IgM levels significantly higher than those of Thal or SCA. This cellular and humoral pattern is clearly distinct from that of the Thal and SCA groups and is strikingly similar to that found in persons with AIDS[29] and AIDS-related conditions.[30] Thus, our findings are consistent with, but expand on, those previous reports concerning immune abnormalities in hemophiliacs.[2-15]

The differences between these three groups strongly suggest that there is something unique about factor concentrate therapy, as opposed to FPRBC therapy, that affects immune function. We have found this to be true even in the high-risk area of New York City, where donors for cellular blood products may be at heightened risk for transmitting AIDS. Furthermore, we found one immune factor, lymphocyte count, stable in both absolute and relative terms for all our blood product recipient groups. We do not know, however, whether numbers or percentage of T-cell subsets have changed over time for these groups. Explanations consistent with our findings include the following possibilities: (1) factor therapy produces a higher antigenic load to its recipients than does RBC therapy, (2) factor concentrate is, by virtue of its biologic nature, immunosuppressive, (3) the etiologic agent for AIDS is transmitted preferentially in noncellular blood fractions and may have been present in some factor concentrate pools before AIDS was recognized as a distinct entity in the general population, and (4) because quantitative donor exposure is much greater for factor users than for RBC recipients, the risk of being exposed to a donor carrying the AIDS agent is greater for hemophiliacs than for other long-term blood product recipients.

We did not find a dose-response relationship between the quantity of factor or of cellular blood product used and laboratory immune abnormalities. In regard to factor dosage, this result is consistent with that of two reports,[5,8] but differs from the results of two other reports[10,15] that found a relationship between factor dosage and T_H/T_S ratio. These latter two reports were from Spain and England; these countries receive US factor concentrate. These differences could therefore represent temporal or quantitative differences between factor distribution to these countries and the United States. Furthermore, the relevance of these researchers' findings to AIDS is unclear. One of these studies showed no direct relationship between factor dosage and numbers of lymphocytes, T_H or T_S,[10] all of which represent immune findings that are more specific for AIDS than is the T_H/T_S ratio.[36] The second study did not report these findings at all.[15]

A number of possible explanations exist for the absence of a dose-response relationship in our patients. First, our blood product information may be inaccurate. We believe this is unlikely because (1) while we have found the recording of factor usage by hemophiliacs and their treatment centers to be highly variable (unpublished data, February through August 1984), records appeared relatively complete in this particular patient population, and (2) we collected three years' worth of blood product data and did our analyses on a yearly and a three-year basis with consistent results. (Cellular blood products were given, not distributed, at the clinics and thus these data are probably more accurate than factor data in any reports using data on home therapy factor dosages.) Second, our study power could have been inadequate for evaluating a dosage effect. This also seems unlikely, given that all r_s values were between $-.200$ and .179. Third, an immunosuppressive effect may be more dependent on short-term than on long-term dosage. Fourth, suppression may occur equally in all persons receiving more than a threshold dosage above which all our factor participants lie and below which all our cellular blood product recipients lie. Fifth, if some of these immune abnormalities represent contact with the AIDS agent, all or none of the factor received by our hemophiliac participants and none of the cellular blood products may have been contaminated with the agent. Sixth, the incubation period for AIDS might differ for different sources of infection. Finally, it may be that the number of different lots (ie, number of different donors) to which a participant was exposed does not correlate well with factor dosage. If this were so, and if donor exposure is the major correlate of risk of exposure to AIDS, one would not expect factor dosage per se to necessarily be correlated with occurrence of AIDS or AIDS-like abnormalities.

In summary, we have found that asymptomatic persons in New York City receiving factor concentrate had immune abnormalities similar to those found in AIDS, while those receiving cellular blood products did not. The relation between these findings and AIDS will become clearer as serologic markers for AIDS become available. The immunosuppressive effects of factor concentrate merit further evaluation using an animal model.

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