

27. Baker SR, Makuch RW, Wolf GT. Preoperative cisplatin and bleomycin therapy in head and neck squamous carcinoma: prognostic factors for tumor response. *Arch Otolaryngol.* 1981; 107:683-9.
28. Albers DS. Perspectives on adjuvant therapy of head and neck cancer. In: Salmon S, Jones S, eds. *Adjuvant therapy of cancer III.* New York: Grune & Stratton, 1981:191-7.
29. Koch H. Biochemical treatment of precancerous oral lesions: the effectiveness of various analogues of retinoic acid. *J Maxillofac Surg.* 1978; 6:59-63.

IMPAIRED CELL-MEDIATED IMMUNITY IN PATIENTS WITH CLASSIC HEMOPHILIA

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THREE cases of *Pneumocystis carinii* pneumonia, two of which were fatal, have recently been observed in patients with severe classic hemophilia who had been treated with lyophilized preparations of antihemophilic factor (factor VIII).¹ Two of the patients also had oral candidiasis, and one was infected with *Mycobacterium avium-intracellulare*. Immunologic studies in the survivors demonstrated poor lymphocyte responsiveness to mitogens, an absolute and relative decrease in helper T cells, and a relative increase in suppressor T cells.

The pathogenesis of this unusual syndrome, which resembles closely the acquired immunodeficiency syndrome (AIDS) that has been detected in homosexual men, Haitians, and intravenous-drug abusers, is not known. The epidemiology of AIDS is suggestive of a blood-borne transmissible agent.² Studies of cell-mediated immunity in patients with AIDS have demonstrated generalized impairment of T-lymphocyte function.³⁻⁷ Our study was designed to examine the immune system of otherwise healthy patients with hemophilia who had received lyophilized antihemophilic-factor concentrates or cryoprecipitates and who might be at risk for the development of AIDS. In this study the results of both lymphocyte-subpopulation surface-marker assays and lymphocyte functional assays were found to be significantly abnormal in the group of patients receiving lyophilized preparations of antihemophilic factor.

METHODS

Subjects

The study population comprised 19 healthy patients with classic hemophilia and 19 age-matched apparently healthy male controls.

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Each subject was studied concurrently with his age-matched control. Of the 19 hemophiliacs, 18 were severely affected (antihemophilic-factor coagulant titer [factor VIII:C] <0.01 U per milliliter), and 1 was moderately affected (antihemophilic-factor titer = 0.04 U per milliliter). Eleven patients, all severely affected, were enrolled in a home-therapy program and had received from 3 to 200 infusions (median, 50 to 200) of lyophilized antihemophilic factor during the year preceding the study. None had a recognized circulating anticoagulant against antihemophilic factor. One patient in this group had had a syndrome resembling idiopathic thrombocytopenic purpura and was studied during remission, while taking no medication, six months after splenectomy. This group of 11 patients was identified as "LYOPH."

Seven of the eight other hemophiliacs had severe disease. Four of them had been treated with cryoprecipitate either alone (three patients) or supplemented twice with lyophilized antihemophilic factor (one patient); one of these patients had a circulating anticoagulant against antihemophilic factor. Four of the eight patients, three of whom had earlier been treated with cryoprecipitate (one had received only fresh frozen plasma), had had no therapy for 20 months or more before our studies; two of them had circulating anticoagulant against antihemophilic factor. This group of eight patients was identified as "CRYO." The mean age (\pm S.D.) of the patients with hemophilia was 32 ± 9 years, and that of the controls was 33 ± 10 years. The CRYO group and their controls (38 ± 10 and 40 ± 10 years, respectively) were older than the LYOPH group and their controls (both 28 ± 5 years) ($P < 0.01$ by Student's *t*-test).

Specimen Collection and Cell Preparation

Venipuncture was performed after informed consent was obtained with the permission of the Committee on Human Experimentation of Case Western Reserve University School of Medicine. Peripheral blood was collected in EDTA for complete blood counts and white-cell differential counts. Heparinized peripheral blood was fractionated by the standard Ficoll-Hypaque gradient-centrifugation technique, and peripheral-blood mononuclear cells were used in assays for lymphocyte markers and function. The investigators performing the assays — with the exception of proliferation assays — were blinded with respect to the identity of patients and controls.

Lymphocyte Surface-Marker Studies

Lymphocyte surface markers were counted by means of an automated flow-cytometer system. The same lots of reagents were used throughout the investigation, and all reagents had been previously standardized for optimal specificity and sensitivity. The accuracy of flow cytometry in this study was verified by performing parallel manual and automated marker studies on the first group of eight subjects; the results were within 5 per cent of one another, regardless of the method used.

A manual counting technique was used to determine the total number of T cells, as assessed by spontaneous sheep-erythrocyte rosette formation (E rosettes), and the total number of B cells, as discerned by the presence of cell-surface immunoglobulin. Total B cells were assayed by direct immunofluorescence, with the use of fluorescein isothiocyanate-labeled F(ab')₂ fragments of goat IgG antitotal human immunoglobulins (Kallestad, Chaska, Minn.). For both these markers at least 300 cells were counted through a microscope equipped for alternating-phase contrast bright-field and epifluorescence (Dialux, Leitz, Rockleigh, N.J.).

A panel of three monoclonal antibodies to T cells were counted with an automated flow-cytometry system (EPICS V, Coulter Electronics, Miami, Fla.). With an argon laser set at 488 nm, 500 mW, the forward light scatter and right-angle green-fluorescence signals were collected as a cytogram for analysis of positive and negative lymphocytes. Positive cells were clearly separated from negative cells, and a total of 10,000 cells were analyzed for each marker examined. The murine monoclonal antibodies used were OKT3, OKT4, and OKT8 (Ortho, Raritan, N.J.). Functionally, OKT3 defines peripheral T cells, OKT4 defines the human helper/inducer T-cell subset, and OKT8 defines the suppressor/cytotoxic T-cell subset.

Absolute numbers of all the above lymphocyte marker-positive subsets were derived from the white-cell and differential counts and were recorded as the number positive per microliter.

Assays of Lymphocyte Function

For assays of lymphocyte proliferation, 10^5 peripheral-blood mononuclear cells suspended in 100 μ l of RPMI-1640 (MA Bio-products, Walkersville, Md.) plus 10 per cent heat-inactivated pooled human serum were cultured in quadruplicate flat-bottomed microtiter wells (Falcon Products, Oxnard, Calif.) with or without mitogen for three days at 37°C in a humidified, 5 per cent carbon dioxide-enriched atmosphere. Purified phytohemagglutinin (Burroughs Wellcome, Research Triangle Park, N.C.) was used at a final concentration of 1 μ g per milliliter, and concanavalin A (Pharmacia, Piscataway, N.J.) at a concentration of 10 μ g per milliliter. These concentrations had been determined previously to be optimal for lymphocyte proliferation. Eighteen hours before harvest, 1 μ Ci of [3 H]thymidine (Scarle, Amersham, England) (specific activity, 5 Ci per millimole) was added to each well. Cells were harvested onto glass-fiber disks, and [3 H]thymidine content was determined by scintillation spectrometry.

Natural-killer activity was assayed as previously described.⁸ In brief, 5×10^3 51 Cr (New England Nuclear, Boston, Mass.)-labeled K562 tumor targets in 150 μ l of RPMI-1640 containing 20 per cent fetal-calf serum (GIBCO, Grand Island, N.Y.) were incubated with peripheral-blood mononuclear cells in triplicate round-bottom microtiter wells (Sarstedt, Princeton, N.J.) for four hours at 37°C in a humidified, 5 per cent carbon dioxide-enriched atmosphere. The microtiter trays were then centrifuged at 150 \times g for 10 minutes, and the radioactivity in a 50- μ l aliquot of supernatant was measured in a gamma counter. The percentage of lysis was calculated according to the following formula:

$$100 \times \frac{\text{cpm (experimental release)} - \text{cpm (spontaneous release)}}{\text{cpm (total release)}}$$

Spontaneous release of 51 Cr from target cells, determined in the presence of medium alone, was always less than 10 per cent of total release; total release was determined after treatment with 1 per cent sodium dodecyl sulfate. Natural-killer activity was assessed for each subject at effector/target ratios of 30:1, 15:1, and 7.5:1 by varying the number of effector cells. Cytotoxicity was linear over this range, and the results are given for effector/target ratios of 30:1.

Linear Discriminant Analysis

In linear discriminant analysis the variables used were the percentage of OKT4 and OKT8 cells and their ratio, total lymphocyte count, responses to phytohemagglutinin and concanavalin A, and natural-killer activity.⁹ The technique used for classification involved "jack-knifing" to eliminate the favorable bias that resulted from evaluating the linear discriminant function in the same subjects who had been used for its computation.¹⁰

RESULTS

Peripheral-Blood Cell Counts

The CRYO group had a higher white-cell count than the control group ($P < 0.01$ by Student's *t*-test) but an equivalent total lymphocyte count, whereas the LYOPH group had more lymphocytes per microliter than the controls ($P < 0.01$ by Student's *t*-test) (Table 1). Both relative and absolute numbers of B lymphocytes, as detected by the presence of cell-surface immunoglobulin, were equivalent in all groups.

The absolute number of T lymphocytes, as measured both by E-rosette formation ($P < 0.05$) and by the presence of OKT3 antigen ($P < 0.001$), was increased in the LYOPH group as compared with controls. The

Table 1. Cell Analyses in Hemophiliacs and Controls.*

	CONTROLS (N = 19)	HEMOPHILIACS (LYOPH N = 11)	HEMOPHILIACS (CRYO N = 8)
White cells/ μ l	4793 \pm 261	5172 \pm 418	6350 \pm 485 †
Lymphocytes/ μ l	1519 \pm 87	1974 \pm 122 ‡	1651 \pm 241
per cent	33 \pm 2	39 \pm 2 †	26 \pm 3 ††
B cells/ μ l	142 \pm 15	185 \pm 33	153 \pm 77
per cent	9.5 \pm 0.9	9.2 \pm 1.4	9.8 \pm 1.1
E-rosette+/ μ l	1195 \pm 65	1555 \pm 136 †	1378 \pm 197
per cent	80 \pm 2	78 \pm 4	84 \pm 3
OKT3/ μ l	1044 \pm 62	1505 \pm 107 ‡	982 \pm 160 ‡
per cent	69 \pm 2	77 \pm 3 ‡	60 \pm 4 ††
OKT4/ μ l	685 \pm 43	745 \pm 73	639 \pm 115
per cent	45 \pm 2	38 \pm 3 †	39 \pm 4
OKT8/ μ l	412 \pm 41	810 \pm 74 ‡	433 \pm 80 †
per cent	27 \pm 2	41 \pm 3 ‡	27 \pm 4 †
OKT4/OKT8 ratio	1.84 \pm 0.16	1.02 \pm 0.17 ‡	1.64 \pm 0.31

*Values are expressed as means \pm S.E.M. White-cell and differential counts were performed on EDTA-treated peripheral blood. All other cell counts were performed on peripheral-blood mononuclear cells obtained by Ficoll-Hypaque density gradient centrifugation. B cells were discerned by the presence of cell-surface immunoglobulin as detected by direct immunofluorescence. Cells bearing the OKT3, OKT4, and OKT8 antigens were counted by flow cytometry (see Methods). LYOPH denotes hemophiliacs treated with lyophilized antithrombotic factor, and CRYO hemophiliacs treated primarily with cryoprecipitate.

†Significantly different from controls, $P < 0.05$ by Student's *t*-test.

‡Significantly different from controls, $P < 0.01$ by Student's *t*-test.

††Significantly different from controls, $P < 0.001$ by Student's *t*-test.

‡‡Significantly different from LYOPH group, $P < 0.01$ by Student's *t*-test.

‡‡‡Significantly different from LYOPH group, $P < 0.005$ by Student's *t*-test.

absolute number of T lymphocytes in the CRYO group did not differ from that in the controls and, as in the controls, was less than the absolute T-lymphocyte counts of the LYOPH group, as determined by the presence of the OKT3 antigen ($P < 0.02$ by Student's *t*-test).

The LYOPH group had a relative decrease in OKT4 helper lymphocytes ($P < 0.05$ by Student's *t*-test), but the absolute numbers of OKT4 cells in both the LYOPH and CRYO groups did not differ from those in the controls. On the other hand, the LYOPH group had both a relative and absolute increase in the number of OKT8 suppressor-cytotoxic lymphocytes ($P < 0.01$ by Student's *t*-test) as compared with both the control and CRYO groups. The OKT8 population in the CRYO group was equivalent to control values. Lastly, the ratio of helper to suppressor cells — the OKT4/OKT8 ratio — was significantly lower in the LYOPH group than in the controls ($P < 0.01$ by Student's *t*-test). This ratio in the CRYO group was equivalent to that in the controls. In the CRYO group, there was no difference between patients who had been treated within the last 20 months and those who had not.

Lymphocyte Proliferative Responses

When compared with the lymphocyte proliferative responses of controls, lymphocytes from the LYOPH group showed diminished proliferation in response to both phytohemagglutinin ($P < 0.01$ by Student's *t*-test) and concanavalin A ($P < 0.025$) (Fig. 1). The proliferative responses of the CRYO group were not different from those of the controls or the LYOPH group.

Natural-Killer Activity

Peripheral-blood mononuclear cells from controls demonstrated more spontaneous cytotoxicity against K562 tumor targets than did those from hemophiliacs who had been treated with lyophilized preparations of antihemophilic factor (Fig. 2). Peripheral-blood mononuclear cells from controls killed 37.8 ± 2.8 per cent (mean \pm S.E.M.) of tumor targets as compared with peripheral-blood mononuclear cells from the LYOPH group, which killed 21.3 ± 3.8 per cent ($P < 0.005$ by Student's *t*-test). The mean killing by peripheral-blood mononuclear cells from the CRYO group (44.4 ± 4.2 per cent) was not different from that of the control group, but it was greater than the killing by peripheral-blood mononuclear cells from the LYOPH group ($P < 0.005$ by Student's *t*-test).

Correlation of Responses

Among the 38 study subjects, 4 had OKT4/OKT8 ratios lower than 0.75. The results of functional assays in these subjects are shown in Table 2. All four were hemophiliacs — three in the LYOPH group and one in the CRYO group. The patient in the CRYO group had not been treated in seven years. The three LYOPH subjects had the three lowest natural-killer-activity scores in this study, each value more than 2.16 standard deviations from the mean for control subjects ($P < 0.05$). The two lowest responses to concanavalin A

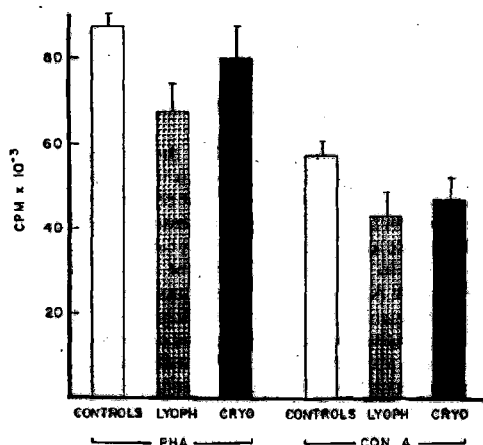


Figure 1. Lymphocyte Proliferation in Hemophiliacs and Controls. The data represent the mean (\pm S.E.M.) proliferative responses to phytohemagglutinin (PHA) ($1 \mu\text{g}$ per milliliter) and concanavalin A (Con A) ($10 \mu\text{g}$ per milliliter), expressed as counts per minute (cpm). The clear bars represent the responses of controls, the shaded bars the responses of hemophiliacs treated with lyophilized antihemophilic factor (LYOPH), and the black bars the responses of hemophiliacs treated primarily with cryoprecipitates (CRYO). When compared with controls, hemophiliacs treated with lyophilized antihemophilic factor had lower proliferative responses to PHA ($P < 0.01$) and Con A ($P < 0.025$). Responses among hemophiliacs treated primarily with cryoprecipitates were not different from those of controls or of the LYOPH group.

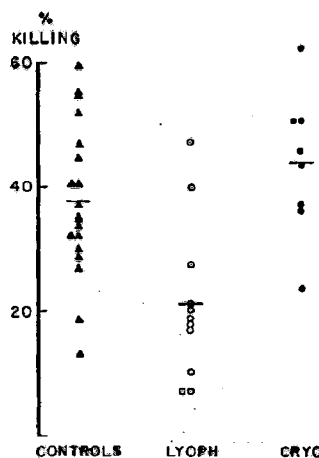


Figure 2. Natural-Killer Activity of Peripheral-Blood Mononuclear Cells Obtained from Controls and Patients with Hemophilia Treated with Lyophilized Antihemophilic Factor (LYOPH) or Primarily with Cryoprecipitates (CRYO). Bars represent the means. Natural-killer activity against K562 tumor targets was lower in the LYOPH group than in the controls ($P < 0.005$ by Student's *t*-test) or the CRYO group ($P < 0.005$). Natural-killer activity in the CRYO group and in the controls was equivalent.

and the lowest response to phytohemagglutinin were also seen in this group of three subjects. In contrast, the subject in the CRYO group had normal natural-killer activity and lymphocyte proliferative responses, all values within one standard deviation of the mean of control values.

Among the LYOPH patients, no correlation could be found between the number of infusions of antihemophilic factor received the year before the study and natural-killer activity, lymphocyte proliferation, the white-cell count, the lymphocyte count, the absolute or relative count of helper and suppressor T cells, or the OKT4/OKT8 ratio.

No significant differences in lymphocyte surface markers or function could be demonstrated between the patients in the CRYO group who had received cryoprecipitate within the past year and those who had not, and although the untreated group tended to have higher OKT4/OKT8 ratios, the differences were not significant.

Linear discriminant analysis demonstrated that at a level that misidentified 2 of 19 normal persons, 8 of the 11 members of the LYOPH group and 2 of the 8 members of the CRYO group were classified as abnormal. The proportion of hemophiliacs who were identified as abnormal was significantly greater in the LYOPH group than in the control group ($P < 0.01$). If a more stringent criterion of separation was used so that no control subjects were misidentified, 6 of the 11 hemophiliacs in the LYOPH group were still classified as abnormal ($P < 0.01$), whereas none of the patients in the CRYO group were so classified.

Table 2. Functional Assays in 4 Subjects with the Lowest OKT4/OKT8 Ratios and in 19 Controls.

	OKT4/ OKT8	NATURAL- KILLER ACTIVITY	PHYTOHEMAG- GLUTININ RESPONSE	CONCANAV- ALIN A RESPONSE
		% killing	counts/minute	
Controls (mean \pm S.D.)	1.84 \pm 0.68	37.8 \pm 12.2	87,342 \pm 15,765	58,548 \pm 13,007
LYOPH subjects				
1	0.55	7.4	53,756	6,476
2	0.63	9.8	16,190	16,941
3	0.66	7.3	65,034	36,058
CRYO subject	0.63	51.1	83,431	48,582

DISCUSSION

These studies demonstrate that some apparently healthy patients with classic hemophilia have abnormalities in cell-mediated immunity. When compared with controls, hemophiliacs treated with lyophilized antihemophilic factor had a relative decrease in helper T cells, a relative and absolute increase in suppressor T cells, and a depressed helper/suppressor T ratio. Functional studies demonstrated depressed natural-killer activity and diminished lymphocyte proliferative responses to the mitogens phytohemagglutinin and concanavalin A. Lymphocyte counts, cell-surface markers, and functions in the eight patients treated primarily with cryoprecipitate did not differ from those in controls. Although the CRYO group was older than the LYOPH group, this age difference was not responsible for the other differences between these groups. When the CRYO group and the LYOPH group were compared individually with their age-matched controls, the findings did not change.

In vitro evidence of impaired cell-mediated immunity has been found in two hemophiliacs with opportunistic infections¹ and in other patients with AIDS.²⁻⁷ The findings in our study differ somewhat from those in these earlier studies. Patients with AIDS and subjects in this study had depressed OKT4/OKT8 ratios,^{6,7} depressed lymphocyte proliferative responses to mitogens,²⁻⁷ and diminished natural-killer activity,⁵ but the magnitude of impairment was much greater in the patients with AIDS than in our subjects with hemophilia. The patients with AIDS generally had an absolute decrease in the number of OKT4 helper cells,^{1,2,6} whereas in hemophiliacs only a relative decrease was observed, with absolute numbers equivalent to those in controls. The patients in this study also had both a relative and absolute increase in OKT8 suppressor/cytotoxic cells, whereas only a relative increase in OKT8 cells has been noted in patients with AIDS.^{1,2,6} Since total lymphocyte counts were normal in the otherwise healthy hemophiliacs, these differences may merely reflect the absolute lymphopenia in patients with AIDS, which would exaggerate the relative lack of OKT4 helpers and normalize the relative increase in OKT8 suppressors. Thus, the phenotypic and functional abnormalities seen in

healthy hemophiliacs may be qualitatively similar to those in patients with AIDS, but quantitatively less severe.

Findings similar to ours have recently been reported among populations of apparently healthy homosexual men. These subjects had a relative^{11,12} and absolute¹² increase in suppressor T cells, a relative and absolute decrease in helper T cells,¹² and a diminished helper/suppressor T ratio.^{11,12} In one study,¹¹ lymphocyte proliferative responses to mitogens were also diminished. A range of cell-mediated immune dysfunction was observed in which healthy homosexuals, homosexuals with hyperplastic lymphadenopathy, and homosexuals with Kaposi's sarcoma had abnormalities of increasing severity.¹¹ In one study,¹² abnormal T-lymphocyte phenotypes correlated with the number of sexual partners per year but not with the use of nitrites; in an earlier study,¹³ abnormal OKT4/OKT8 ratios in healthy homosexuals had been detected primarily among nitrite users. Whether the immune dysfunctions observed in vitro in healthy homosexuals or in the hemophiliacs described in this study will predispose to the development of clinical immunosuppression and opportunistic infections is not clear.

What are the possible explanations for the abnormalities in cell-mediated immunity among patients with hemophilia? Conceivably, abnormal cell-mediated immunity is part of the genetic hemophilia disorder, but this explanation is unlikely for several reasons. Until recently, hemophiliacs did not appear to be at greater risk for infections, other than those attributable to the administration of blood products. In addition, hemophiliacs treated only with cryoprecipitates had no demonstrable functional abnormalities in cell-mediated immunity.

A more likely possibility is that the immune dysfunction is acquired. Active infection with hepatitis B virus is probably not responsible, since none of the 11 patients in the LYOPH group had demonstrable hepatitis B surface antigenemia. The cause of the immunosuppression in this population is not known; among patients with AIDS, however, epidemiologic evidence would implicate a blood-borne pathogen. Whether or not this putative immunosuppressive agent is responsible for the abnormalities in cell-mediated immunity that we have observed in healthy hemophiliacs and for the opportunistic infections recently described in this population remains to be determined.

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REFERENCES

1. *Pneumocystis carinii* pneumonia among persons with hemophilia A. *Morbidity and Mortality Weekly Report*. 1982; 31:365-7.
2. Marx JL. New disease baffles medical community. *Science*. 1982; 217:618-21.

3. Gottlieb MS, Schroff R, Schanker HM, et al. *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *N Engl J Med.* 1981; 305:1425-31.
4. Masur H, Michelis MA, Greene JB, et al. An outbreak of community-acquired *Pneumocystis carinii* pneumonia: initial manifestation of cellular immune dysfunction. *N Engl J Med.* 1981; 305:1431-8.
5. Stegall FP, Lopez C, Hammer GS, et al. Severe acquired immunodeficiency in male homosexuals, manifested by chronic perianal ulcerative herpes simplex lesions. *N Engl J Med.* 1981; 305:1439-44.
6. Friedman-Kien A, Laubenstein LJ, Rubinstein P, et al. Disseminated Kaposi's sarcoma in homosexual men. *Ann Intern Med.* 1982; 96:693-700.
7. Mildvan D, Mathur U, Enlow RW, et al. Opportunistic infections and immune deficiency in homosexual men. *Ann Intern Med.* 1982; 96:700-4.
8. Brovall C, Schacter B. Radiation sensitivity of human natural killer cell activity: control by x-linked genes. *J Immunol.* 1981; 126:2236-9.
9. Lachenbruch PA. Discriminant analysis. New York: Macmillan, 1975.
10. Stepwise discriminant analysis (Program BMDP7M, BMDP Statistical Software). Berkeley, Calif.: University of California Press, 1981:519-37.
11. Stahl RE, Friedman-Kien A, Dubin R, Marmor M, Zolla-Pazner S. Immunologic abnormalities in homosexual men: relationship to Kaposi's sarcoma. *Am J Med.* 1982; 73:171-8.
12. Kornfeld H, Vande Stouwe RA, Lange M, Reddy MM, Orieo MH. T-lymphocyte subpopulations in homosexual men. *N Engl J Med.* 1982; 307:729-31.
13. Goedert JJ, Neuland CY, Wallen WC, et al. Amyl nitrite may alter T lymphocytes in homosexual men. *Lancet.* 1982; i:412-6.

T-LYMPHOCYTE SUBPOPULATIONS IN PATIENTS WITH CLASSIC HEMOPHILIA TREATED WITH CRYOPRECIPITATE AND LYOPHILIZED CONCENTRATES

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PATIENTS with the acquired immunodeficiency syndrome (AIDS) include male homosexuals, intravenous drug abusers, and Haitian immigrants to the United States.¹⁻³ Approximately half the patients have contracted *Pneumocystis carinii* pneumonia, a third Kaposi's sarcoma, and 10 per cent concurrent Kaposi's sarcoma and *P. carinii* pneumonia. The mortality rate approaches 40 per cent. Persistent generalized lymphadenopathy and diffuse undifferentiated non-Hodgkin's lymphoma have also been reported in homosexual males and are considered part of the AIDS.^{4,5} Nine patients (six with Kaposi's sarcoma and three with *P. carinii* pneumonia) were found to have had sexual contact with other AIDS patients.⁶

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Among homosexual patients, studies of immunologic functions have demonstrated lymphopenia, cutaneous anergy, reduced helper T-lymphocyte (T4) subpopulations, increased suppressor T-lymphocyte (T8) subpopulations, inverted helper/suppressor T-lymphocyte (T4/T8) ratios, an abnormal lymphocyte response to mitogen stimulation, decreased natural-killer-cell activity, and paradoxically, hypergammaglobulinemia.⁷⁻¹² Altered T-lymphocyte subpopulations were also found in asymptomatic homosexuals, although the abnormalities were less severe than those seen in homosexuals with Kaposi's sarcoma or *P. carinii* pneumonia.^{13,14} In one study an increased frequency of the HLA-DR5 phenotype was found in homosexual men with Kaposi's sarcoma.¹⁰

Recently, three cases of *P. carinii* pneumonia were diagnosed in patients with hemophilia A.¹⁵ Although these patients had hemophilia, they had not received immunosuppressive therapy and did not have other underlying diseases. They were heterosexuals with no history of intravenous drug abuse. Their immunologic evaluation revealed dysfunction of cellular immunity (inverted T4/T8 ratio, an impaired lymphocyte response to mitogen stimulation, and a reduced number of circulating T cells). All had been treated with large doses of commercially prepared lyophilized factor VIII concentrates as part of the treatment of their coagulation defect. Two of the three received transfusions for prophylaxis almost daily, and the third received them every four to five days. One patient used concentrates prepared by six different manufacturers; another, concentrates prepared by five manufacturers; and a third, concentrates prepared by three or four. None used the same lot of factor VIII concentrate. Another case of *P. carinii* pneumonia and one of cryptosporidiosis occurring in patients with hemophilia A are currently being investigated (Evatt BL: personal communication).

The clustering of AIDS in patients with common sexual contacts and the occurrence of *P. carinii* pneumonia among users of factor VIII concentrates have led to speculation that AIDS may be transmitted to patients with hemophilia through factor VIII infusion.¹⁶ To evaluate this possibility we performed immunologic studies on healthy patients with hemophilia treated either with cryoprecipitate obtained from volunteer blood donors or with commercially prepared, lyophilized factor VIII concentrates. Eight of 22 patients had abnormal T4/T8 ratios. None of the cryoprecipitate users and 57 per cent of the users of commercially prepared lyophilized concentrates had abnormal T4/T8 ratios ($P < 0.003$).

METHODS

Patients with hemophilia who were being followed in the Comprehensive Hemophilia Program of the Great Lakes Hemophilia Foundation were divided into three groups: those being treated only with cryoprecipitate prepared from plasma obtained from volunteer blood donors at the Blood Center of Southeastern Wisconsin, those using high amounts of commercially prepared factor VIII concen-