

T-Lymphocyte Subpopulation Abnormalities in Apparently Healthy Patients with Hemophilia

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T-lymphocyte populations in 12 apparently healthy heterosexual adult patients with hemophilia have been examined to ascertain if these patients have abnormalities in their lymphocyte subpopulations similar to those in homosexual men, Haitian refugees, and narcotic addicts. A striking reduction in the helper to suppressor cell ratio ($[0.86 \pm 0.14]:1$) was found in 9 of the 12 patients. These abnormalities were similar to those previously described in two patients with hemophilia critically ill with *Pneumocystis carinii* pneumonia, and in homosexual men. The abnormality in the ratio of helper to suppressor T cells may be related to the continual exposure of patients with hemophilia to commercial clotting factor concentrates. However, these patients with abnormal T-lymphocyte subpopulations remain healthy. At present there is insufficient evidence to advocate a change in therapeutic practices in patients with hemophilia.

A RECENT OUTBREAK of opportunistic infections, immunologic disorders, and malignancies has been identified in homosexual men, Haitian refugees, and narcotic addicts (1-7). Evaluation of lymphocytes from homosexual men has identified a decrease in T-cell numbers and function, suggesting immunologic dysfunction in the pathogenesis of these diseases (8, 9). A characteristic feature of the altered cellular immune functions is a reduced ratio of leu-3 positive (helper/inducer) to leu-2 (suppressor/cytotoxic) T-lymphocytes. It has been suggested that these abnormalities are a result of continued exposure to sexually transmitted viral infections, particularly cytomegalovirus infections.

The Centers for Disease Control has identified an outbreak of *Pneumocystis carinii* pneumonia in adult patients with hemophilia and questioned whether they represent another patient group with disordered immune function and enhanced risk for infections and malignancy (10). Two of the patients studied had evidence of cellular immune dysfunction with inverted T-helper/T-suppressor cell ratios. As with the homosexual men, patients with hemophilia may be continually exposed to viral antigens because they receive parenteral infusions of coagulation factors that may contain viral particles (11). As a result of these preliminary observations, we evaluated T-lymphocyte populations in 12 apparently healthy, heterosexual adult patients with hemophilia to ascertain if they have abnormalities in their lymphocyte subpopulations similar to those in homosexual men.

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Methods

Heparinized blood was obtained after the patients gave informed consent. The mononuclear cell layer was separated from the blood by ficoll-hypaque gradient separation (12). This cell layer was washed twice in Hanks Balanced Salt Solution, then resuspended in RPMI-1640 (KC Biological, Lenexa, Kansas) with 5% heat-inactivated fetal calf serum at a concentration of 5×10^6 cells/mL.

Lymphocytes binding either Leu-3_a or Leu-2_a murine monoclonal antibodies (Becton-Dickinson, Sunnyvale, California) were identified by light microscopy using alkaline-phosphatase conjugated goat anti-mouse immunoglobulin as a developing antibody (Cappel Laboratories, Cochranville, Pennsylvania) as previously described (12). Briefly, 0.2 mL samples of the cell suspensions were incubated with 0.005 mL of each of the monoclonal antibodies for 30 minutes at 4 °C. After incubation, the cell suspension was washed twice with media at 4 °C, and then 0.03 mL of the developing antibody was added to the cell pellet and incubated for 30 minutes at 4 °C. After incubation, the cells were washed twice in cold media. Cells binding the monoclonal antibodies were stained by exposing the alkaline phosphatase on the cells to 0.1% Naphthol AS-TR phosphoric acid and 0.03% Fast Violet B Salt (Ig indicator, Sigma B lymphocyte kit; Sigma Chemical Co., St. Louis, Missouri) as previously described (13). This suspension was incubated at 37 °C for 30 minutes, then washed twice with media and centrifuged. To identify monocytes using a peroxidase stain, the pellet was mixed with 1.0 mL media, 0.005 mL of 0.12% H₂O₂ solution, and 0.025 mL peroxidase indicator (Sigma B lymphocyte kit) and incubated for 1 minute at room temperature. The suspension was centrifuged and the pellet was resuspended in 0.025 mL media. Using these techniques, monocytes stain black, positive lymphocytes stain red, and negative lymphocytes do not stain.

The data are expressed as the percentage of lymphocytes that bind either Leu-3_a or Leu-2_a monoclonal antibodies. Two hundred lymphocytes were counted. Coagulation assays were done as previously described (14, 15).

Patients

Twelve consecutive patients with hemophilia were evaluated (Table 1). Eleven patients had severe disease with plasma levels of less than 0.01 U/mL of factor VIII or factor IX, and lifelong recurrent spontaneous hemarthroses. Patient 10 had a factor IX level of 0.04 U/mL and a moderately severe bleeding disorder.

Frequency of treatment differed from weekly to more than monthly. Numerous lots of clotting factor concentrate were used. Only Patient 4 had never received commercial lyophilized clotting factor concentrates. All patients were heterosexual and born in the United States. No patient used illicit intravenous narcotics.

Patients 1 and 9, had circulating anticoagulants (inhibitors) to factor VIII. Four patients had splenomegaly and three, hepatomegaly. Lymphadenopathy was not found in any patients. Associated medical conditions included mild alcohol abuse in two patients, mild congestive heart failure in one patient, and asthma in one patient. The patients with congestive heart fail-

Table 1. Characteristics of Patients with Hemophilia

Patient	Deficient Factor	Presence of Inhibitor	Splenomegaly	Other Medical Conditions	Aspartate Transaminase*	Cytomegalovirus Titer†
					IU/L	
1	VIII	Yes	No	None	27	< 1:8
2	VIII	No	Present	Alcoholism	168	1:32
3	VIII	No	No	None	40	< 1:8
4	VIII	No	No	None	19	< 1:8
5	VIII	No	No	None	57	< 1:8
6	VIII	No	No	None	40	< 1:8
7	IX	No	No	Alcoholism	220	< 1:8
8	VIII	No	No	Asthma	24	< 1:8
9	VIII	Yes	Present	None	48	< 1:8
10	IX	No	Present	Mild congestive heart failure	163	< 1:8
11	VIII	No	Present	None	60	< 1:8
12	VIII	No	No	None	137	1:32

* Normal, 7.5-40 IU/L.
 † Normal, less than 1:8.

ure and asthma were not receiving medications at the time of evaluation. The patients ranged in age from 16 to 71 years (median, 28 years). All of the patients have had liver function studies at least once a year, as well as laboratory studies to detect infections with hepatitis B virus, cytomegalovirus, and toxoplasmosis.

Ten patients had cytomegalovirus titers of less than 1:8 and two patients had titers of 1:32 by complement fixation. All patients had toxoplasmosis titers of less than 1:64, by indirect hemagglutination, except Patient 7, who had a toxoplasmosis titer of 1:256 for 3 years. All patients had negative test results for hepatitis B surface antigen but all had elevated titers of antibodies directed against hepatitis B surface antigen. Seven patients had aspartate transaminase levels 1.2 to 5.5 times normal. Four of these patients had a remote history of overt hepatitis with elevated levels of bilirubin. The ratio of helper to suppressor T-cells was also concurrently determined in 10 age-matched normal men.

Results

In the 12 patients with hemophilia, the ratio of Leu-3_a positive (helper/inducer) cells to Leu-2_a positive (suppressor/cytotoxic) cells was $[0.86 \pm 0.14]$ (mean \pm SEM):1 (Figure 1). Age- and sex-matched non-hemophilic adult men had a ratio of 1.96 ± 0.24 (mean \pm SD):1 ($p < 0.001$, compared to patients with hemophilia). All normal patients had a ratio of Leu-3_a positive cells to Leu-2_a positive cells greater than 1.4:1. There was a striking separation of the patients with hemophilia into two distinct groups. Patients 4, 9, and 10 had normal Leu-3_a/Leu-2_a cell ratios whereas the remaining nine patients had a ratio of less than 0.82:1 (Figure 1). In patients with normal Leu-3_a/Leu-2_a cell ratios, the absolute numbers of both Leu-3_a and Leu-2_a were within the normal range (data not shown). In patients with abnormal ratios, the absolute numbers of Leu-3_a cells were decreased whereas the absolute numbers of Leu-2_a cells were either in the normal range or markedly increased (data not shown).

Discussion

A striking abnormality in the ratio of helper to suppressor T cells was found in 9 of 12 heterosexual adult patients with hemophilia. These abnormalities were simi-

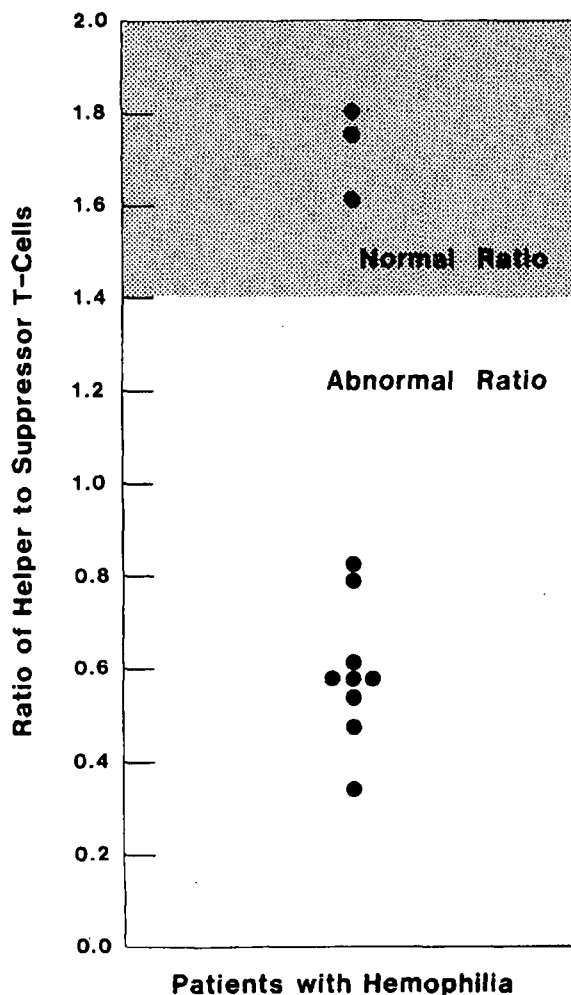


Figure 1. Ratio of Leu-3_a positive (helper/inducer) lymphocytes to Leu-2_a positive (cytotoxic/suppressor) lymphocytes in patients with hemophilia. Normal patients have a Leu-3_a to Leu-2_a ratio of $[1.96 \pm 0.28]$ (mean \pm SD):1. All ratios are greater than 1.4 to 1 (shaded area).

lar to those previously described in two patients with hemophilia who were critically ill with *Pneumocystis carinii* pneumonia, and in homosexual men. In our patients, the abnormality in the ratio of helper to suppressor T cells appeared to be related to continual exposure to commercial clotting factor concentrates, as all patients with these abnormalities were treated approximately once a week with the commercial products. In marked contrast, none of the three patients with normal subpopulations of T cells had ongoing frequent exposure to these products. Patient 4 had normal subpopulations and had never used commercial clotting factor concentrates, although he had over 100 lifetime exposure days to whole blood, plasma, and individual donor cryoprecipitate. A second patient (Patient 9) has an acquired circulating anticoagulant to factor VIII and has been treated every 4 to 6 months with prothrombin complex concentrates exclusively since 1979. Patient 10 has had two extensive exposures to factor IX concentrates during a total hip replacement, but fewer than 50 total lifetime exposure days.

All of our patients had serologic evidence for antecedent exposure to hepatitis B antigen. These patients may be similar to other patients with hemophilia who have had immunofluorescent evidence for persistent viral infection of hepatocytes seen on liver biopsy specimens despite the presence of circulating antibody to the agent of hepatitis B (16). Although none of our patients have had recent evidence of hepatitis other than mildly abnormal liver tests (Table 1), persistent viral infection may have caused the disordered ratio of T-helper/T-suppressor cells in these patients. Alternatively, these patients may be continually exposed to hepatitis B antigen associated with infusion of the clotting factor concentrates. In addition, T-cell abnormalities similar to those described in these patients have also been described in patients with acute and chronic hepatitis B infections (8).

It has been suggested that chronic exposure to cytomegalovirus or nitrates may be responsible for the alterations in T-cell populations in homosexual men (17). Although these agents may be responsible for the T-cell abnormalities in homosexuals, it is unlikely that these agent caused defects in patients with hemophilia because none of these patients had been exposed to nitrates and all had stable antibody titers to cytomegalovirus, equal to or less than 1:32.

The presence of an abnormal ratio of helper to suppressor T-cells in these patients is of uncertain significance, and this observation needs to be confirmed with functional assays of help and suppression. In addition, since the establishment of the Great Plains Regional Hemophilia Center at the University of Iowa in 1976, no cases of pneumonia or chronic infection have occurred in our patients. A single patient has died of malignancy (disseminated melanoma). Whether patients with these T-cell defects are at increased risk for development of

malignancy has yet to be substantiated. Reports from the literature, however, suggest that patients with congenital bleeding disorders have a prevalence of malignant disease similar to the general population (18). At this time, there is insufficient evidence to advocate a change in therapeutic practices in these patients. However, additional patients with hemophilia need to be evaluated to ascertain if the magnitude of exposure to clotting factor concentrates is associated with an increased incidence of malignancy or opportunistic infections.

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