

Immunologic Studies in Asymptomatic Hemophilia Patients

RELATIONSHIP TO ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS)

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ABSTRACT Asymptomatic hemophilia patients receiving Factor VIII concentrate were found to have normal natural killer (NK) cells and B cells, and an inverted T helper/suppressor ratio due to an increase in cells of T suppressor phenotype. In contrast, a hemophilia patient with acquired immune deficiency syndrome (AIDS) exhibited nonfunctional NK cells, low B cells, and an inverted T helper/suppressor ratio due to very low numbers of T helper cells. Hemophilia patients on cryoprecipitate therapy exhibited normal immune parameters. A high percentage of hemophilia patients on both treatments had antibody to hepatitis B virus. The isolated finding of elevated levels of T suppressor cells in hemophilia patients receiving Factor VIII concentrate has not been recognized as an early indicator of impending AIDS, and longitudinal studies will be required to determine its clinical significance.

INTRODUCTION

An acquired immune deficiency syndrome (AIDS)¹ has been associated with an unprecedented outbreak of

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¹Abbreviations used in this paper: AIDS, acquired immune deficiency syndrome; CMV, cytomegalovirus; EBNA,

Pneumocystis carinii pneumonia, other opportunistic infections and Kaposi's sarcoma among homosexual men (1, 2), drug addicts of both sexes (3, 4), and Haitian refugees (2, 5). Seven patients with hemophilia A have recently been reported to have the clinical and laboratory features of AIDS (6, 7); all of these individuals had received Factor VIII concentrates.

We have examined immunological parameters in healthy hemophilia patients, normal individuals, and a previously reported hemophilia patient with AIDS (7). The emphasis was on two groups of hemophilia patients, one treated with Factor VIII concentrate and the other with cryoprecipitate, in an attempt to define possible risk factors involved in developing AIDS.

METHODS

Study population. 51 asymptomatic individuals (25 ± 18.1 yr of age) with hemophilia A were evaluated at the Birmingham Hemophilia Program of the Alabama State Crippled Children Rehabilitation Service. All were receiving Factor VIII concentrate on a home management program. Another individual with hemophilia and AIDS (7) who was receiving Factor VIII concentrate therapy was also evaluated. Blood samples from 13 asymptomatic patients (38.8 ± 14.1 yr of age) on cryoprecipitate therapy from the Hemophilia Center of Western New York in Buffalo were also analyzed. 39 normal individuals (33.7 ± 11.6 yr; 21 males and 18 females) from Birmingham were included in the

Epstein-Barr nuclear antigens; EBV, Epstein-Barr virus; HSV, herpes simplex virus; NK cells, natural killer cells; VCA, viral capsid antigens.

study. Age-matched male controls served as blood donors for the studies of natural killer (NK) cells.

Immunological studies. Serum immunoglobulin levels were determined by a fluorometric assay (8). Mononuclear cells isolated from heparinized blood on Ficoll-Hypaque gradients were examined for expression of cell surface antigens by indirect immunofluorescence using an affinity purified fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulin reagent and the following monoclonal antibodies: Leu 4 (pan T), Leu 3 (T helper), Leu 2 (T suppressor-cytotoxic), HNK-1 or Leu 7 (NK cells), MMA or Leu-M1 (myeloid/monocytic) (Becton Dickinson & Co., Mountain View, CA), and HB-2 (pan B) (9, 10). Positive cells were enumerated by a fluorescence-activated cell sorter (FACS IV, Becton Dickinson & Co.) (10). Two-color immunofluorescence analysis was used in determining the percentage of HNK-1⁺ cells expressing Leu 4 and Leu 2 markers (11). NK cell activity was assessed by ⁵¹Cr release from K562 target cells (9).

Viral antibodies. IgG antibodies to Epstein-Barr nuclear antigens (EBNA) and viral capsid antigens (VCA) were determined by established immunofluorescence procedures (12). Antibodies to cytomegalovirus (CMV) were measured by an enzyme-linked immunoassay (12; M. A. Bioproducts, Walkersville, MD); antibodies to herpes simplex virus (HSV) by neutralization (12); and antibodies to hepatitis B surface and core antigens by radioimmunoassay (AUSAB and CORAB kits, Abbott Laboratories, North Chicago, IL). For each assay, control and patient specimens were run simultaneously.

Statistical analysis. The analysis of variance was used to compare lymphocyte subpopulation distributions in the single AIDS patient and the two groups of hemophilia patients with those found in healthy controls. Rank order statistics were used to compare antibody titers. Linear regression analysis was used to correlate percent HNK-1⁺ cells and percent ⁵¹Cr release assays for patients and controls.

RESULTS

Leukocyte subpopulation studies. The hemophilia patient with AIDS was very lymphopenic with significant reductions in all lymphocyte subpopulations measured (Table I, $P < 0.01$). In contrast, no reduc-

tions in the numbers of total leukocytes, lymphocytes, T cells, B cells, NK cells, or monocytes were seen in the two treatment groups of hemophilia patients compared with healthy controls. However, a significant increase ($P < 0.004$) in the number of Leu 2⁺ T cells and a corresponding increase in total T cells were noted in hemophilia patients receiving Factor VIII concentrate therapy (Fig. 1). This resulted in a decreased T helper/suppressor ratio for this group of patients (1.2 ± 0.6 vs. 2.1 ± 0.8 control values; $P < 0.004$), with a ratio < 1 in 26 of 51 individuals. Patients on cryoprecipitate therapy had normal numbers of T cell subsets and normal Leu 3⁺/Leu 2⁺ ratios (2.1 ± 0.7). The hemophilia patient with AIDS had very low numbers of T helper cells and a mean helper/suppressor ratio of 0.16 ± 0.05 .

NK cell studies. NK function, assessed by a ⁵¹Cr release cytotoxicity assay, was normal for patients receiving either Factor VIII concentrate or cryoprecipitate therapy, and normal proportions of HNK-1⁺ cells expressed the T cell antigen, Leu 4 (Table II). For both groups of healthy hemophilia patients, there was a good correlation between the frequency of HNK-1⁺ cells and NK function (Factor VIII concentrate, $r = 0.72$; cryoprecipitate, $r = 0.75$). The patient with AIDS showed depressed NK function, 83–100% coexpression of HNK-1 and Leu 4, and 88% coexpression of HNK-1 and Leu 2, features associated with NK cell immaturity (11).

Viral antibody studies. The percentage of seropositive hemophilia patients was not significantly different from controls when antibodies to CMV, EBV, and HSV were measured, but a high percentage ($> 60\%$) of patients on both therapies had antibodies to hepatitis B surface and core antigens. When compared with controls, the geometric mean titers deter-

TABLE I
Evaluation of Circulating Leukocyte Subpopulations
in Individuals with Hemophilia A

Populations of blood cells (mm ³)	Hemophilia and AIDS (n = 1)*	Healthy hemophilia patients receiving		Healthy controls (n = 39)
		Factor VIII concentrate (n = 51)	Cryoprecipitate (n = 13)	
Total leukocytes	4,333±412‡	6,341±284	6,630±364	5,974±205
Lymphocytes	683±164	2,091±131	1,850±264	1,744±70
T cells (Leu-4 ⁺)	305±79	1,575±89	1,011±100	1,185±55
B cells (HB-2 ⁺)	30±10	275±24	360±38	286±40
NK Cells (Leu-7 ⁺)	123±53	316±38	309±46	272±37
Monocytes (MMA ⁺)	307±75	478±27	435±32	427±41

* Data determined from five samples obtained over a 5-mo period.

‡ All values expressed as mean±SE.

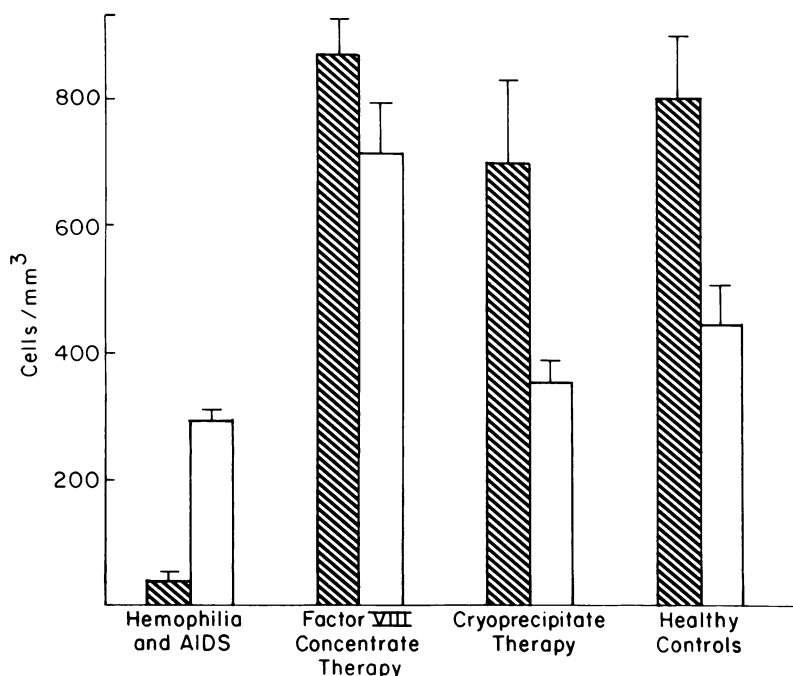


FIGURE 1 Absolute numbers (cells per cubic millimeter) of circulating Leu-3⁺ T helper (▨) and Leu-2⁺ T suppressor cells (□) in hemophilia patients and healthy controls. Brackets denote standard error of the mean.

mined for CMV were significantly elevated in both hemophilia groups ($P < 0.03$; Fig. 2), whereas EBNA and VCA values were not significantly elevated.

Serum immunoglobulins. The levels of IgG ($1,540 \pm 221$), IgM (149 ± 19), and IgA (226 ± 45) determined for 27 of the 51 hemophilia patients receiving Factor VIII concentrate therapy were normal except for slight elevation of IgA in two patients. The IgG ($1,305 \pm 100$), IgM (144 ± 18), and IgA (208 ± 21) levels determined for the 13 patients on cryoprecipitate were normal. The hemophilia patient with AIDS had normal

levels of IgG ($1,697 \pm 102$) and IgM (225 ± 44) but persistently high IgA (786 ± 55); immunoelectrophoresis revealed that the IgA was polyclonal.

DISCUSSION

Increasing evidence for a blood-borne etiologic agent for AIDS and its sporadic occurrence in hemophilia patients has caused alarm in these individuals. The use of large donor pools for preparation of Factor VIII concentrate and single donors for cryoprecipitate

TABLE II
Analysis of Functional and Phenotypic Features of NK Cells
in Individuals with Hemophilia A

	Hemophilia and AIDS (n = 1)*	Healthy hemophilia patients receiving		Healthy controls (n = 21)
		Factor VIII concentrate (n = 24)	Cryoprecipitate (n = 13)	
HNK-1 ⁺ (Leu-7) (%)	18 ± 4 †	16 ± 2	11 ± 2	12 ± 2
$\frac{\text{Leu4}^+ \text{HNK}^+}{\text{HNK}^+}$ (%)	94 ± 3	45 ± 4	49 ± 6	41 ± 5
⁵¹ Cr release § (%)	11 ± 3	35 ± 4	37 ± 4	44 ± 3

* Data determined from four samples obtained over a 4-mo period.

† All values expressed as mean ± SE.

§ Target/effecter cell ratio, 1:10.

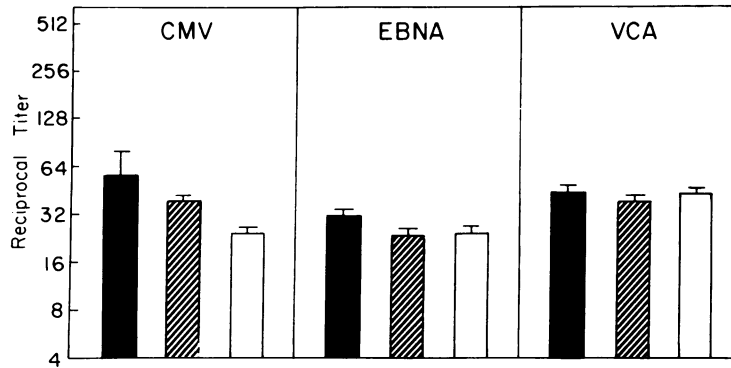


FIGURE 2 Geometric mean titers \pm standard error for CMV, EBNA, and VCA from hemophilia patients on Factor VIII concentrate ($n = 33$, ■), cryoprecipitate ($n = 13$, ▨), and normal age-matched controls ($n = 20$, □).

preparations could thus have significant risk implications. Here, we have demonstrated the frequent occurrence of a reversal of the T helper/suppressor ratio in healthy hemophilia patients who have received Factor VIII concentrates. However, this was not due to a reduction in T cells with the helper Leu 3⁺ phenotype, as has been noted in homosexuals (1), other hemophilia patients with AIDS (Fig. 1; references 6, 7), and in asymptomatic homosexuals (13). Instead, a substantial increase was found in the numbers of T cells with the suppressor Leu 2⁺ phenotype. This proved to be an isolated finding in the hemophilia patients receiving Factor VIII concentrate. No correlation was found between the amount of Factor VIII concentrate used and the extent of the T cell abnormality.²

Normal circulating levels of monocytes, B cells, immunoglobulins, and NK cells were observed in all of the healthy hemophilia patients. In contrast, the hemophilia patient with AIDS was lymphopenic with very low numbers of circulating T cells, B cells (three-fold increase in IgA), and NK cells. Most of the HNK-1⁺ cells in this patient coexpressed the Leu 4 and Leu 2 antigen markers and apparently lacked NK function. The expression of Leu 2 antigen by the HNK-1⁺ cells, a phenotype associated with immature NK cells (11), contributed to the dramatic reversal of the "T helper/suppressor" ratio in this patient, and may indicate the existence of an even greater T cell deficit than initially appreciated.

Viruses like CMV, EBV, and HBV, which are known to contaminate blood products, can cause transient alterations of lymphocyte function and reversal of the helper/suppressor ratio (14-16). We noted that viral titers for CMV were elevated in healthy hemophilia patients belonging to both treatment groups, and most

of these individuals had antibodies to hepatitis B virus antigens. However, normal antibody levels to EBV nuclear (EBNA) and capsular (VCA) antigens were found in individuals receiving either Factor VIII concentrate or cryoprecipitate therapy.

The results of the present studies emphasize the importance of determining both absolute and relative numbers of cells with a variety of phenotypic markers in evaluating lymphocyte subpopulations in patients at risk of the AIDS syndrome. The clinical significance of the increase in cells of the T suppressor phenotype that we observed in some healthy hemophilia patients is unclear. Longitudinal studies, presently underway, are needed to determine whether or not these hemophilia patients receiving Factor VIII concentrate are at a higher risk for developing AIDS than are those receiving cryoprecipitate therapy.

Note added in proof. In two other recent studies (17, 18), a similar imbalance of circulating T lymphocyte subpopulations was noted in hemophilia patients receiving Factor VIII concentrates, but not those treated with cryoprecipitates.

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² Landay, A. Unpublished observations.

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