CONCISE REPORT

Lymphadenopathy-Associated Virus Antibodies and T Cells in Hemophiliacs Treated With Cryoprecipitate or Concentrate

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Evidence for exposure to lymphadenopathy-associated virus (LAV) was investigated in 48 patients with hemophilia, 15 of whom had been treated exclusively with single-donor cryoprecipitate. The prevalence of antibodies to LAV in all patients was 53% in 1983 and 63% in 1984, while in patients treated only with cryoprecipitate, the prevalence was 31% in 1983 and 40% in 1984. Patients treated with any concentrate had a seroprevalence of 65% in 1983 and 77% in 1984. Seropositive patients were more likely to have a significant reduction in the ratio of helper to suppressor T cells, absolute numbers of helper T cells, and T cell function in vitro. Seven of 18 patients who were seronegative in 1983 had seroconverted by 1984. The relative risk of seroconversion for patients using any concentrate since 1981 compared with those using cryoprecipitate only was 3.9 (P = .04). Nevertheless, the rate of conversion in the latter group was 18% per year.

ABNORMALITIES in T cell subsets and T cell function have been reported in patients with hemophilia A and B.[1-8] Although the clinical significance of these observations is unclear, hemophiliacs are known to be at high risk for developing the acquired immunodeficiency syndrome (AIDS),[9] presumably as a consequence of infection with the lymphadenopathy-associated virus (LAV),[10] also known as HTLV III.[11] Recent reports have identified antibodies to this virus in otherwise healthy hemophiliacs.[11,12]

The current study evaluated T cell subsets and T cell function in hemophilic patients receiving commercial concentrates or single-donor cryoprecipitate in relation to the presence or absence of anti-LAV antibodies. Patients were reevaluated after 12 months, allowing us to determine whether there were any changes in LAV antibody prevalence or immune status.

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MATERIALS AND METHODS

Subjects. The study included 48 patients with moderate or severe factor VIII (FVIII) or factor IX (FIX) deficiency treated at the Puget Sound Blood Center, Seattle. Informed consent was obtained according to institutional guidelines. Forty-three were studied in Janaury 1983 and 41 in January 1984. Of 42 patients with FVIII deficiency, 14 were moderately affected (1% to 5% FVIII) and 27 were severely affected (<1% FVIII). The other six patients had severe FIX deficiency. Patients were grouped according to treatment (Table 1). Median age of the patients was 28 years and was comparable for all treatment groups. Controls consisted of healthy volunteers, with a median age of 36 years (range, 13 to 47), with no known risk factors for AIDS.

T cell subsets and function. T helper (T_h) cells and T suppressor (T_s) cells were identified by indirect immunofluorescence using monoclonal antibodies 66.1 to identify the T_h marker, CD4 (T4, Leu-3 equivalent), and 51.1 to identify the T_s marker, CD8 (T8 or Leu-2 equivalent).[8] T cell function was assessed by testing mitogen-induced proliferation of peripheral blood mononuclear cells cultured with 20% heat-inactivated human serum in microtest plates (5×10^4 cells per 100 μL per well). Phytohemagglutinin (PHA) was added in final concentrations of 0.25, 0.5, and 2.5 μg/mL, concanavalin A (Con A) in final concentrations of 20, 40, and 80 μg/mL, and pokeweed mitogen (PWM) in final dilutions of 1:50, 1:125, and 1:200. Cultures stimulated with PHA and Con A were incubated for three days and those with PWM for five days at 37 °C with 5% CO_2. Proliferation was measured as cpm of tritiated thymidine incorporation following a three-hour pulse label.

LAV serology. Anti-LAV antibodies were assayed by Western blot[14] using purified detergent-lysed LAV. LAV-containing supernatants were kindly provided by Dr Luc Montagnier, Institut Pasteur, Paris.[10] Seropositivity was defined as a reaction with any of the viral antigens p18, p25, or p41.

Table 1. Patient Characteristics

Type of Therapy*	No. of Patients†	Amount of Replacement Therapy‡	
		Cryoprecipitate	Concentrate
I. Cryoprecipitate only	15 (8)	57 (8-193)	0
II. Cryoprecipitate + concentrate	19 (13)	104 (27-252)	1 (<1-68)
III. Concentrate only	14 (13)	0	127 (39-323)
Total	48		

*Cryoprecipitate was prepared from single volunteer donor units.[13] FVIII or FIX concentrate was obtained commercially.

†Five patients included in the 1984 evaluation were not studied in 1983, and seven patients studied in 1983 were not available in 1984. Numbers in parentheses indicate numbers of patients severely affected.

‡Numbers represent units $\times 10^3$ per year. One donor unit of cryoprecipitate is equivalent to 100 FVIII units; one bottle of concentrate is equivalent to 1,000 FVIII or FIX units.

Table 2. Anti-LAV Antibody in Patients With Hemophilia

Type of Replacement Therapy	Positive LAV Serology 1983	Positive LAV Serology 1984	Rate of Seroconversion*
I. Cryoprecipitate only	5/16 (31%)	6/15 (40%)	2/10 (20%)
II. Cryoprecipitate + concentrate	10/15 (67%)	10/14 (71%)	5/8 (62%)
III. Concentrate only	7/11 (64%)	10/12 (83%)	
Total	22/42 (53%)	26/41 (63%)	7/18 (39%)

*Eighteen patients evaluated in both 1983 and 1984 were seronegative in 1983.

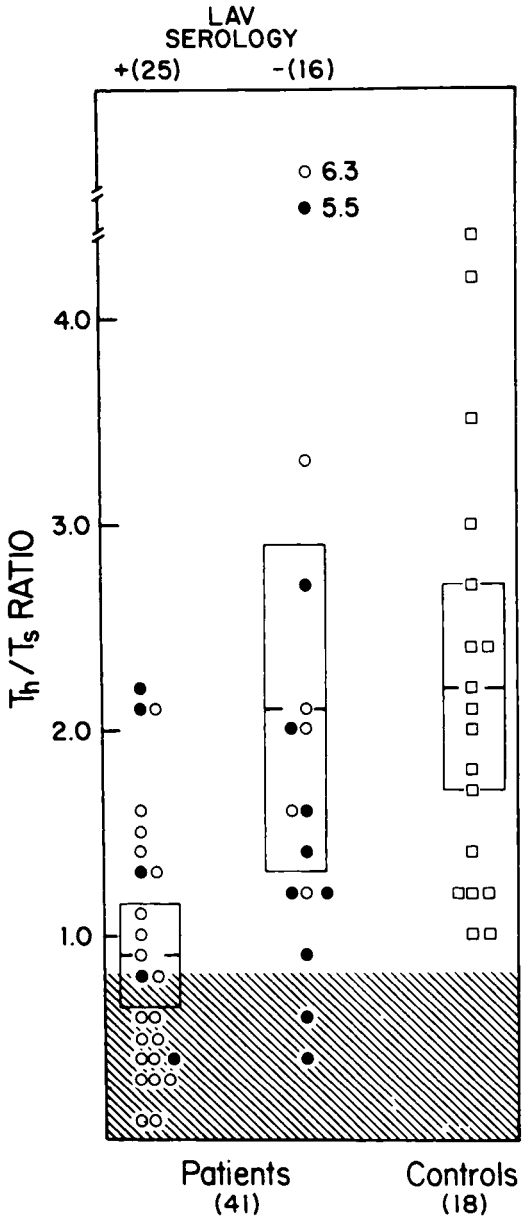

Fig 1. T_h/T_s ratios in hemophilic patients according to presence or absence of anti-LAV antibody (●, patients receiving cryoprecipitate only; ○, patients receiving any concentrate; □, controls). Means and 95% confidence intervals are indicated by windows. The shaded area represents the abnormally low range as determined by testing healthy normal controls (n = 60).

RESULTS

LAV serology. Antibodies to LAV were detected in 53% of patients in 1983 and 63% in 1984 (Table 2). Seroprevalence was significantly greater in patients treated with concentrate (65% in 1983, 77% in 1984) compared to patients treated only with cryoprecipitate (31% in 1983, 40% in 1984) ($P < .02$, chi-square test). Of the 36 patients evaluated in both 1983 and 1984, 18 were seronegative in 1983. Ten of the 18 seronegative patients had received only cryoprecipitate; an additional seronegative patient received only cryoprecipitate since January 1981. Of these 11 patients, two (18%) seroconverted. Of the remaining seven patients, five (71%) seroconverted. Thus, the relative risk of seroconversion for patients using any concentrate since January 1981 was 3.9 ($P = .04$, Fisher's exact test).

Relationship between LAV serology and T cell subsets. T_h/T_s ratios were significantly lower in seropositive patients compared to seronegative patients in 1984 (median, 0.8 *v* 1.6; $P < .002$, Mann-Whitney U test) (Fig 1). A similar association between LAV serology and T_h/T_s ratio was also

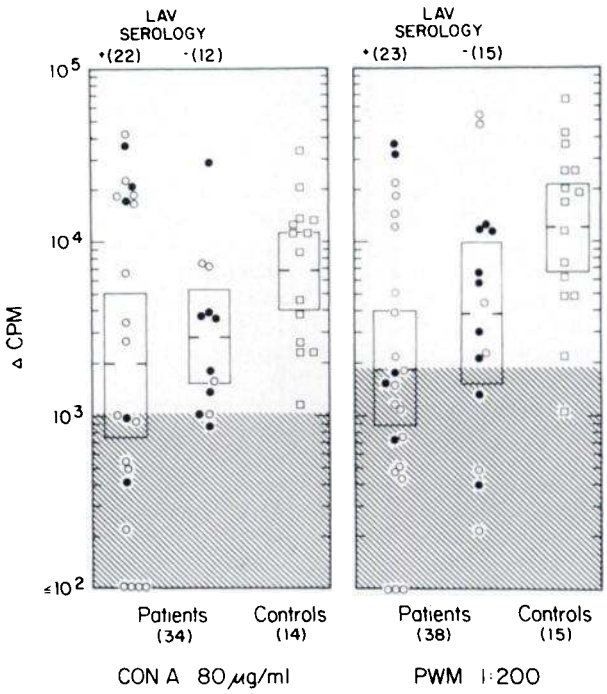

Fig 2. T cell proliferation in hemophilic patients in response to stimulation with Con A (80 μg/mL) or PWM (final dilution, 1:200). Shaded area represents the abnormally low range of response as determined by testing healthy normal controls (mean − 2 SD).

observed in 1983 (data not shown). In the seven patients who seroconverted between 1983 and 1984, however, no significant change was seen in the T_h/T_s ratio. The T_h/T_s ratio also remained stable in 19 patients who were already seropositive in 1983.

Relationship between LAV serology and T cell function. Responses to PWM, but not Con A, were significantly lower in seropositive patients than controls ($P = .005$, Mann-Whitney U test) (Fig 2). Low responses to PWM or Con A (< control mean − 2 SD) were significantly more frequent in seropositive patients than controls (odds ratio >21, $\chi^2 > 10.1$; $P < .01$). Responses in seronegative patients, however, did not differ significantly from those in seropositive patients or in controls. When patients were stratified according to type of replacement treatment, no significant association was seen between LAV serology and T cell response.

Clinical status. Thirty-six of 48 patients first evaluated in January 1983 were available for evaluation in January 1984, and all but four remained well. One 10-year-old seropositive patient in group II developed molluscum contagiosum, eosinophilia, and intermittent abdominal pain. A 35-year-old seropositive patient in group IV had immune thrombocytopenia requiring splenectomy. Two patients died, one after trauma and the other with a ruptured aneurysm.

DISCUSSION

Antibodies to LAV were detected in hemophilic patients treated only with cryoprecipitate (40% in 1984) as well as in patients treated with concentrate (77% in 1984). The rate of seroconversion between 1983 and 1984 was greater for patients receiving any concentrate (18% *v* 71%) since January 1981. Thus, although the prevalence of antibody and the relative risk of seroconversion (3.9) was greater in the concentrate group, these data also indicate that the transfusion of large amounts of cryoprecipitate collected from healthy volunteer donors but not screened for anti-LAV antibodies can be associated with substantial risk of exposure to infectious virus.

A significant association was seen between anti-LAV antibodies and abnormalities in the T_h/T_s ratios and T cell function, suggesting that alterations in T cell subsets and T cell function can be related in transfusion-related exposure to LAV. However, abnormal T_h/T_s ratios and T cell responses were not detected in all patients exposed to LAV, and progressive changes were not observed in patients who seroconverted during the course of study. With the exception of two individuals, patients also remained well and free of unusual infections or other clincal evidence of immunodeficiency.

Whether the immune status of these patients will continue to be stable, even assuming no further exposure to LAV, is unknown. Screening blood donors for anti-LAV antibodies will presumably decrease the risk of AIDS transmission. Additional measures, however, such as heat inactivation,[15] continue to represent important alternatives for preventing infection in patients exposed to blood products from a large number of donors.

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