Human T-Lymphotropic Retrovirus Type III/Lymphadenopathy-Associated Virus Antibody

Association With Hemophiliacs' Immune Status and Blood Component Usage

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• **We studied the human T-lymphotropic retrovirus type III/lymphadenopathy-associated virus (HTLV-III/LAV) antibody status of 234 factor VIII concentrate recipients, 36 factor IX concentrate recipients, 69 long-term recipients of frozen packed red blood cells, and 47 persons not receiving routine transfusion therapy. Factor VIII concentrate recipients had a significantly higher rate of seropositivity (74%) than any other group. Factor IX concentrate recipients had a significantly higher rate (39%) than recipients of frozen packed red blood cells (4%) or nontransfused persons (4%). In factor VIII concentrate recipients, HTLV-III/LAV seropositivity was significantly associated with more severe hemophilia, greater factor dosage, elevated immunoglobulin and immune complex levels, lower T-helper lymphocyte numbers, and lower ratios of T-helper to T-suppressor lymphocytes. For factor IX concentrate recipients, seropositivity was associated with more severe hemophilia. Antibody-positive factor IX concentrate recipients had a lower rate of seropositivity to HTLV-III/LAV p41 membrane antigen than did antibody-positive factor VIII concentrate recipients, but factor VIII and factor IX concentrate recipients had similar rates of seropositivity to core antigens. We conclude that both factor VIII and factor IX concentrates may transmit HTLV-III/LAV. For factor VIII recipients, HTLV-III/LAV seropositivity is associated with altered immune test results.**

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PERSONS with hemophilia in the United States are at heightened risk for the acquired immunodeficiency syndrome (AIDS).[1] As of Jan 30, 1985, forty-eight persons with severe hemophilia A, three with moderate hemophilia A, five with mild hemophilia A, and three with hemophilia B have been reported to the Centers for Disease Control (CDC) as meeting CDC AIDS criteria. Two persons with severe hemophilia A and one with hemophilia B had other risk factors for AIDS. Using denominators extrapolated from a 1975 survey,[2] we estimate that persons with severe hemophilia A are at fourfold to fivefold higher risk of CDC-defined AIDS than are persons with mild or moderate hemophilia A or B. We suspect that this elevated risk is due to their relatively greater use of factor VIII concentrate products and that factor VIII concentrate may transmit the AIDS agent. This hypothesis is supported by the fact that at least nine patients with AIDS having hemophilia as their only risk factor for AIDS received no blood products other than factor VIII concentrate (personal communications, patients' physicians).

Two newly reported isolates are prototype strains of the human retrovirus thought to be the etiologic agent of AIDS: human T-lymphotropic retrovirus type III (HTLV-III)[3,4] and lymphadenopathy-associated virus (LAV).[5,6] The virus appears to be cytotoxic for a subset of T-helper (T_H) lymphocytes.[7] Seropositivity to HTLV-III/LAV antigens has not been found to be associated with abnormal T-lymphocyte subset numbers or function in two studies[8,9] but was associated with low T_H lymphocyte numbers in a third study.[10]

In this article we evaluate and compare recipients of factor VIII concentrates, factor IX concentrates, and

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frozen red blood cells and persons not receiving chronic transfusion therapy to address the following issues concerning AIDS and blood product recipients. First, what is the seroprevalence of antibody to HTLV-III/LAV antigens in various hemophilia A populations? Second, do factor IX recipients show serologic evidence of contact with HTLV-III/LAV, and, if so, does it appear to be related to their use of factor? Third, does LAV seroprevalence differ for factor recipients and persons receiving frozen packed red blood cells in New York City, an area with a high incidence of AIDS? Fourth, is there any association between HTLV-III/LAV seropositivity and the quantities of factor VIII, factor IX, and/or other blood products used? Last, is there any association between HTLV-III/LAV seropositivity and results in any of a variety of immunologic laboratory tests?

PARTICIPANTS AND METHODS

Participants

New York City.—Participants were enrolled from clinic patient rosters between April and November 1983. Details of patient selection and evaluation are given elsewhere.[11] Briefly, serum was obtained from 49 patients with hemophilia A who had received 100,000 or more units of factor VIII from two treatment centers in New York City in the 12 years preceding their enrollment in our study. Only seven had received other blood products in the previous five years, for isolated bleeding incidents. These two centers treat approximately 40% of the hemophiliac patients in that city. We also evaluated 43 persons with β-thalassemia from a center that treats 67% of the transfusion-dependent thalassemic patients in New York City. These persons received frozen packed red blood cells to maintain a hemoglobin level of greater than 11 mg/dL. Twenty-six patients with sickle cell anemia were enrolled from three centers in New York City that use routine frozen packed red blood cell transfusion therapy. Blood specimens were also obtained from 18 healthy New York City health care personnel who volunteered to be controls for laboratory testing.

Georgia.—Serum samples were collected in November and December 1982 from 49 patients with hemophilia A and two with hemophilia B enrolled in the Hemophilia of Georgia home care treatment program, from ten persons receiving renal dialysis, and from 19 persons with chronic active hepatitis. Five persons with chronic active hepatitis were homosexual men. Sample collections were repeated in August 1983 through January 1984 for 23 patients with hemophilia A and two with hemophilia B.

Other US Participants.—Sixty-two hemophilia A and 30 hemophilia B participants were enrolled from February through August 1984 as an unexposed cohort in a matched study of recipients of lots of factor VIII and factor IX that were voluntarily withdrawn by the manufacturers because a donor contributing to the lots later developed AIDS. Unexposed individuals were clinically well except for their hematologic disorder and were randomly chosen and matched to exposed individuals on the basis of age, sex, and type/dose of factor used.

Vienna.—Specimens were collected between August 1982 and June 1983 in Vienna, Austria, from 74 hemophilia A and four hemophilia B participants. All patients had been treated with factor VIII or factor IX concentrates in the hemophilia treatment center at the University of Vienna for several years, in large part with material produced in Vienna but containing a variable (10% to 90%) portion of plasma from North American donors. Immunologic evaluation of most of the Austrian participants has been presented in detail elsewhere.[12]

All Participants.—No participants had received a combination of factors VIII and IX in the preceding three years. All participants were clinically well and without symptoms of AIDS at the time of evaluation and, except for the five men with chronic active hepatitis noted above, reported having no risk factors for AIDS other than hemophilia. This information was based on standardized confidential questionnaires completed in private by the US participants and on information supplied by the collaborating physicians for the Viennese participants. All but one of the factor recipients were male; 94% were white. Median ages were as follows: factor VIII recipients, 27 years (range, 1 to 66 years); factor IX recipients, 22 years (6 to 59 years); thalassemic patients, 19 years (10 to 36 years); sickle cell anemia patients, 18 years (7 to 57 years); dialysis patients, 55 years (41 to 76 years); chronic active hepatitis patients, 43 years (21 to 67 years); and healthy controls, 30 years (27 to 50 years). Factor VIII recipients used a median of 56,301 factor units in the previous year (0 to 553,250 units); factor IX recipients used a median of 29,080 units (0 to 106,320 units). Thalassemic patients used a median of 36 units of frozen packed red blood cells in the previous year (12 to 60 units) and a median of 108 units in the previous three years (30 to 170 units). Sickle cell anemia patients used a median of 17 units of frozen packed red blood cells in the previous year (0 to 60 units) and 41 units in the previous three years (11 to 142 units).

Methods

Serum specimens were tested for antibody to HTLV-III/LAV by Western blot analysis. Virus was provided by the Institute Pasteur, Paris. Lymphadenopathy-associated virus was separated by ultracentrifugation of culture supernates from LAV-infected, phytohemagglutinin-stimulated human lymphocytes' through a 30% w/w sucrose cushion (80,000 g for one hour). The pellets were dissolved in 0.01M TRIS, pH 8.0, containing 1% sodium lavryl sulfate, 0.25 mg/mL of bromphenol blue, 10% glycerol, and 5% 2-mercaptoethanol and were heated at 60 °C for 30 minutes. Western blots were performed by the method of Tsang et al.[13] Serum specimens were tested at a 1:100 dilution, and banding patterns were compared with those of a known positive control serum. This technique detects 18kd, 25kd, 32kd, 41kd, and 110kd viral proteins, but we have detected the 32kd and 110kd antigens only in association with the other proteins; therefore, serologic reactions with any combination of the 18kd, 25kd, and 41kd proteins of LAV were scored as positive.

In all but Viennese participants, lymphocyte subpopulations were quantitated by indirect immunofluorescence on a fluorescence-activated cell sorter (FACS IV), with commercial monoclonal antibodies (OKT3 for T-cells, OKT4 for T_H/inducer cells, and OKT8 for T-suppressor (T_S)/cytotoxic cells and fluorescein-conjugated anti-mouse immunoglobulin (CDC).[14,15] Immunoglobulins G, A, and M were quantitated by nephelometry. Methods used for immunologic testing for Viennese participants have been described elsewhere.[12] Briefly, lymphocyte subpopulations were quantitated on a fluorescence-activated cell sorter with Leu 4a (Pan T-cells), Leu 3a (T_H/inducer cells) and Leu 2a (T_S/cytotoxic cells). Immunoglobulins were determined by radial immunodiffusion (Mancini) using Partigen plates. Normal laboratory values obtained by these techniques did not differ from those obtained at the CDC.[12]

Lymphocyte transformation responses were quantitated by a micromethod, using the mitogens phytohemagglutinin (n=245), concanavalin A (n=245), and pokeweed (n=211).[16]

The staphylococcal binding assay and the iodine 125 C1q binding assay were performed as previously described.[17,18]

Statistical Analysis

"Positive" or "negative" results were analyzed using Fisher's exact test (two-tailed) or χ^2 analysis. The significance of the relationship between disease severity and HTLV-III/LAV seroprevalence was determined by the test for a linear trend in proportions.[19] Immunologic and sero-

Table 1.—Human T-Lymphotropic Virus Type III/ Lymphadenopathy-Associated Virus Seroprevalence in Persons With Hemophilia, by Location and Type of Factor Used

	Factor VIII, % (No. Positive/No. Tested)	Factor IX, % (No. Positive/No. Tested)
New York	88 (43/49)	. . .
Georgia	61 (30/49)	0 (0/2)
Other locations, United States	87 (54/62)*	40 (12/30)
Vienna	62 (46/74)†	50 (2/4)
Total	74 (173/234)	39 (14/36)

*Seroprevalence rate is for first Georgia sample and did not differ significantly from that of the second Georgia sample (18/23). Rates for the three US groups were not statistically different from one another.
†P=.007 for difference between rate for Viennese and US factor VIII recipients.

Table 2.—Human T-Lymphotropic Virus Type III/Lymphadenopathy-Associated Virus Seroprevalence, by Participant Group

	% Positive	No. Positive/No. Tested
Factor VIII recipients*	74	173/234
Factor IX recipients†	39	14/36
Thalassemia	7	3/43
Sickle cell anemia	0	0/26
Others not receiving factor‡	4	2/47

*P<.0001 for difference between factor VIII recipients and factor IX recipients.
†P<.0001 for difference between factor IX recipients and recipients of cellular blood products, ie, thalassemics and sickle cell anemics; P=.0001 for difference between factor IX recipients and all persons not receiving factor (Fisher's exact test).
‡These included ten persons receiving renal dialysis, 19 persons with chronic active hepatitis, and 18 healthy heterosexual male volunteers from New York City.

Table 3.—Human T-Lymphotropic Virus Type III/Lymphadenopathy-Associated Virus Seroprevalence in Persons With Hemophilia, by Severity of Disease and Type of Factor Used

	% Positive (No. Positive/No. Tested)†	
Severity*	Factor VIII‡ (n=140)	Factor IX§ (n=20)
Mild	29 (2/7)	0 (0/4)
Moderate	62 (10/16)	20 (2/10)
Severe	78 (91/117)	83 (5/6)

*Mild, more than 5% factor activity; moderate, 1% to 5% factor activity; severe, less than 1% factor activity.
†This information was available for 140 factor VIII and 20 factor IX recipients.
‡P=.0026.
§P=.0028.

logic results were compared between selected groups using the Wilcoxon rank-sum test.[20] Spearman's rank correlation coefficient (r_s) was used to measure the strength of association between disease severity and factor dosage in the previous year.[20] Logistic regression analysis was used to investigate the relation of (1) HTLV-III/LAV serologic status to age for factor VIII recipients, taking the effect of factor dosage into account, and (2) cellular immune tests to HTLV-III/LAV serologic status, taking into account factor dosage, age, and disease severity for factor VIII recipients.[21,22] The dependent variables in the latter cases were dichotomized using the laboratory median values for factor VIII recipient participants; independent variables were treated categorically. The significant level for all statistical analyses was .05.

RESULTS

Seroprevalence of HTLV-III/LAV antigens in persons with hemophilia did not vary significantly in different regions of the United States; 79% of US factor VIII and 37% of US factor IX users were seropositive to at least one HTLV-III/LAV antigen (Table 1). Seroprevalence for the Austrian factor VIII users was significantly lower than that for the entire United States (P=.007), but it did not differ significantly from that of the first Georgia sample (61%), collected in a similar time period. (Seroprevalence of the first and second Georgia samples also did not differ significantly from one another.) Seroprevalence for factor IX recipients did not vary by location.

Seroprevalence of HTLV-III/LAV in factor VIII recipients varied significantly between age groups: of those aged 9 years or younger, 43% were positive; 10 to 44 years, 80%; and 45 years or older, 42% (P=.0001). (Since factor VIII dosage in the previous year was correlated with age, we performed a logistic regression analysis including both age and factor dosage as independent variables and found that these differences in HTLV-III/LAV seroprevalence by age group remained.) Seroprevalence of HTLV-III/LAV in factor IX recipients did not vary significantly with age.

Factor VIII and factor IX recipient groups did not differ significantly in sex, race, or age. Significantly more factor VIII than factor IX recipients were HTLV-III/LAV antibody positive (Ab+) (P<.0001, Table 2). However, seroprevalence in factor IX recipients was significantly higher than that in recipients of frozen packed red blood cells (P<.0001) or in all persons not receiving factor (P=.0001). Of the Ab+ factor IX recipients, none had received blood products other than factor IX in the previous year; one had received 4 units of red blood cells and 2 units of plasma in the previous five years.

The three seropositive thalassemic patients had used 27, 40, and 40 units of frozen packed red blood cells in the previous year and 79, 109, and 108 units in the previous three years. Their usage was therefore quite close to the median usage for the thalassemic group. Two persons with chronic active hepatitis were seropositive. One was a homosexual man with more than 30 sexual partners in the previous year; the other was a 51-year-old white married man who reported having used no blood products in the previous three years and denied risk factors for AIDS.

Seroprevalence of HTLV-III/LAV increased with increasing disease severity for both factor VIII and factor IX recipients (Table 3). Disease severity was positively associated with factor usage in the previous year (factor VIII: r_s=.21, P=.02; factor IX: r_s=.49, P=.04). Antibody-positive factor VIII recipients used significantly more units than did the antibody-negative (Ab−) recipients (Table 4). Antibody-positive factor IX recip-

Table 4.—Dosage of Factor, by Human T-Lymphotropic Virus Type III/Lymphadenopathy-Associated Virus Antibody Status and Type of Factor Used

Factor Units	Factor VIII		Factor IX	
	Antibody Positive	Antibody Negative	Antibody Positive	Antibody Negative
Previous year				
Median	70,500	20,038	47,010	26,440
Range	2,340-553,250	0-212,000	11,120-106,320	0-55,570
No.	118	42	7	10
P	<.0001		NS*	
Yearly average†				
Median	111,101	36,855	...	...
Range	11,079-481,420	1,499-229,530	...	...
No.	53	10	...	...
P	.0078		...	...

*NS indicates not significant.
†Yearly average for participants providing three years of factor information.

Table 5.—Lymphocyte Populations and Ratios of T-Helper to T-Suppressor Lymphocytes of Hemophiliac Participants, by Human T-Lymphotropic Virus Type III/Lymphadenopathy-Associated Virus Antibody Status and Type of Factor Used

	Factor VIII		Factor IX*	
	Antibody Positive	Antibody Negative	Antibody Positive	Antibody Negative
Total lymphocytes/cu mm†				
Median	1,782	2,160	2,108	2,397
Range	532-4,794	740-9,546	752-3,312	893-4,444
No.	167	57	13	21
P	.0090		NS‡	
T-helper lymphocytes/cu mm§				
Median	664	1,037.5	719	991
Range	170-4,458	259-3,150	135-1,764	420-2,205
No.	165	56	13	21
P	<.0001		NS	
T-suppressor lymphocytes/cu mm‖				
Median	678	606	665	653
Range	218-3,403	135-1,754	316-1,259	196-1,398
No.	165	56	13	21
P	NS		NS	
T-helper to T-suppressor lymphocyte ratio¶				
Median	0.8	1.3	1.4	1.6
Range	0.1-2.4	0.4-3.9	0.3-2.0	0.7-3.1
No.	167	57	13	22
P	<.0001		NS	

*Differences between factor VIII and factor IX antibody-positive recipients not significant.
†Laboratory normal range, 1,050 to 3,118/cu mm.
‡NS indicates not significant.
§Laboratory normal range, 408 to 1,583/cu mm.
‖Laboratory normal range, 190 to 820/cu mm.
¶Laboratory normal range, 1.0 to 3.9/cu mm.

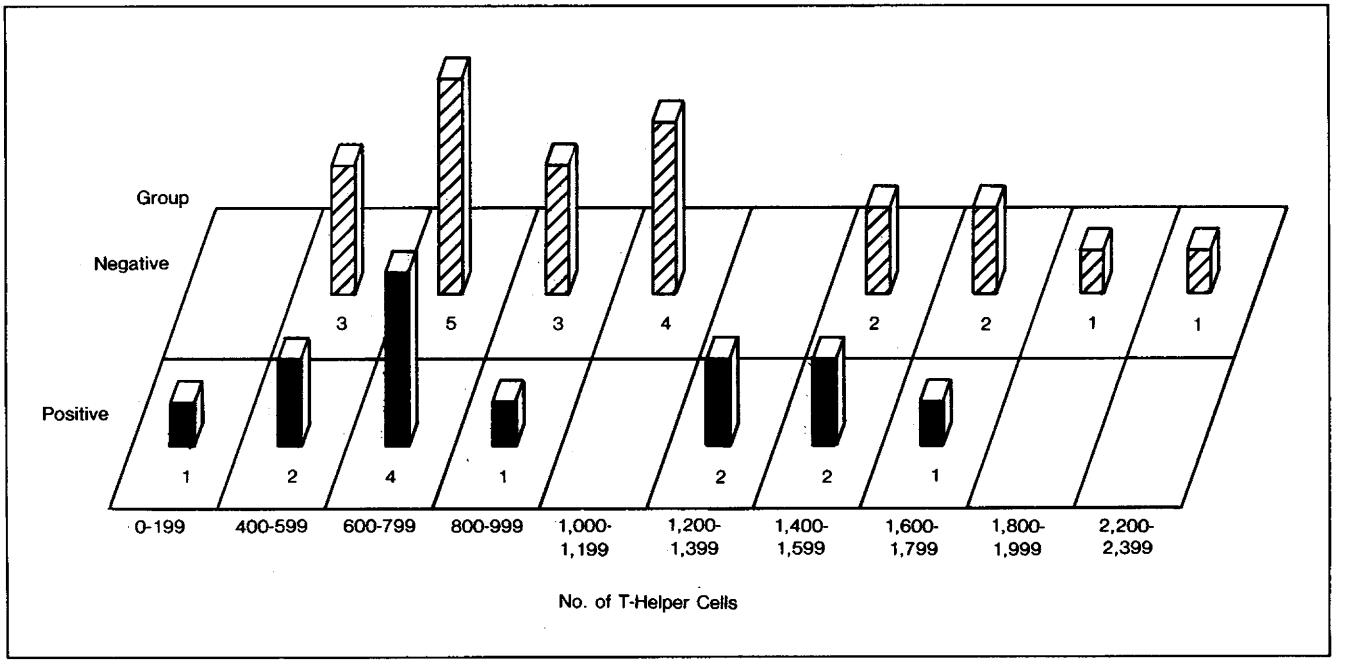
ients used more units than did Ab− factor IX recipients, but this difference was not significant. (These trends were found for both the New York City and other participants.)

Antibody-positive factor VIII recipients had significantly lower total lymphocyte counts, T_H lymphocyte numbers, and T_H/T_S ratios than did Ab− factor VIII recipients (Table 5). The associations between HTLV-III/LAV serological status and cellular immune tests persisted after adjusting for age and factor dosage by logistic regression. Antibody-positive factor VIII recipients had significantly lower T_H/T_S ratios than did Ab+ factor IX recipients (P=.015); other differences were not significant. Differences between Ab+ and Ab− factor IX recipients were not significant (Table 5). Unlike factor VIII recipients, factor IX recipients had distributions of T_H lymphocytes for both Ab+ and Ab− that were potentially bimodal in nature (Figure). Factor IX recipients were therefore separated into four subgroups, based on the midpoint of the T_H interval in which no individuals fell: Ab− persons with less than 1,150 T_H lymphocytes per cubic millimeter, Ab− persons with 1,150/cu mm or more, Ab+ persons with less than 1,150/cu mm, and Ab+ persons with 1,150/cu mm or more. The Ab− subgroups were compared with each other, as were the Ab+ sugbroups. These comparisons indicated no significant difference between subgroups in immunoglobulin levels, mitogen responses, immune complexes, or factor usage; however, numbers were quite small for all three comparisons (data not shown).

Antibody-positive factor VIII recipients had significantly higher levels of IgG, IgA, and immune complexes than did Ab− factor VIII recipients (Table 6). Antibody-positive factor VIII and Ab+ factor IX recipients did not differ significantly in immunoglobulin levels, but Ab+ factor VIII recipients had significantly higher levels of immune complexes (C1q binding assay, P=.014; staphylococcal binding assay, P=.003). Antibody-positive and Ab− factor IX recipients did not differ significantly in regard to immunoglobulin or immune complex levels. Antibody-positive and Ab− factor VIII and factor IX recipients did not differ in responses to stimulation with mitogens (data not shown).

Antibody-positive factor VIII and factor IX recipients had similar seroprevalence of HTLV-III/LAV p18 core antigen (87% and 86%, respectively) and to p25 core antigen (99% and 93%, respectively). However, seroprevalence of p41 surface antigen was significantly less for factor IX than for factor VIII recipients (42% and 80%, P=.005 by Fisher's exact test) (Table 7). We compared, within the factor VIII and factor IX recipient groups, persons who were seropositive to p41 antigen with those who were seropositive to p18 and/or p25 but not to p41 antigen.

There were no significant differences in immune test results between these groups. Furthermore, the seropositive factor IX recipient subgroups (Figure) did not differ significantly in



Distribution of T-helper lymphocyte numbers, by human T-lymphotropic virus type III/lymphadenopathy-associated virus antibody status for factor IX recipients.

seroprevalence to p41 (T_H lymphocytes: <1,150/cu mm, 35% seropositive; ≥1,150/cu mm, 45%).

COMMENT

Most of the participants receiving factor VIII concentrate manufactured all or in part from US blood donations are seropositive to one or more HTLV-III/LAV antigens. The lower rate of seropositivity in Viennese participants may be due to the lower proportion of US donors for their product, the earlier timing of their sample, and/or a time lag in shipment of US AIDS-contaminated plasma to foreign countries and is consistent with the seroprevalence rate found in a group of Danish hemophiliacs treated in part with US donor material.[23] Despite the difference between Viennese and US factor recipients, the extent of seropositivity in all the factor VIII recipients studied is striking. The statistically significant associations of hemophilia severity and of factor VIII dosage with HTLV-III/LAV seropositivity strongly implicate factor VIII concentrates in HTLV-III/LAV transmission, as do the occurrence of AIDS in nine US persons receiving only factor VIII concentrates[24] and the finding by Melbye et al[23] of an association between seropositivity and commercial concentrate consumption.

A lower proportion of factor IX than factor VIII recipients were seropositive to HTLV-III/LAV antigens. However, their rate of seropositivity was significantly higher than that of frozen packed red blood cell recipients from an area of high AIDS prevalence and that of other participants not receiving factor products.* Only one of these 14 seropositive factor IX recipients had received any blood product other than factor concentrate in the previous five years. Thus, the 39% HTLV-III/LAV seroprevalence rate in factor IX recipients is highly suggestive that factor IX concentrate may transmit HTLV-III/LAV antigens, if not whole virus. Furthermore, the significant association of seropositivitiy with severity of disease indirectly implicates factor IX concentrates in HTLV-III/LAV transmission. This is consistent with the occurrence of AIDS in three persons with hemophilia B; however, two of these persons had risk factors for AIDS other than hemophilia.[1] It is also consistent with the reported isolation of LAV from two siblings with hemophilia B.[6] With a 61% rate of seronegativity, factor IX recipients represent a prime target population for AIDS prevention, eg, through the use of heat-treated products.[24-26]

When we began these analyses, we hoped they would resolve a major concern surrounding the theorized role of HTLV-III/LAV as the cause of AIDS, ie, if HTLV-III/LAV is tropic and cytotoxic for T_H lymphocytes,[7] as might be reasonably expected of a putative AIDS agent, it was worrisome that serologic studies involving groups at risk for AIDS showed no association between HTLV-III/LAV serologic status and any immune test.[8,9] This lack of association could represent insensitivity in the serologic tests used, but we postulated that it might instead be due to inadequate study power and/or a mixed population of persons within the Ab+ and Ab− groups. There are at least three possible ways in which these populations might be considered "mixed."

First, since concentrates may be immunosuppressive,[27,28] due to the antigenic nature of factor itself, the cellular debris in concentrates, or non-HTLV-III/LAV viruses transmissible in factor (eg, hepatitis B virus; non-A, non-B hepatitis viruses;

*It should be emphasized that these recipients of frozen packed red blood cells were enrolled from an area in which blood donors may have had a heightened rate of infectivity for HTLV-III/LAV. Furthermore, the recipients of frozen packed red blood cells evaluated in this study had received large volumes of blood products. Thus, the seroprevalence rate for this group should not be considered representative of that expected for other cellular blood product recipients.

Table 6.—Immunoglobulin and Immune Complex Levels of Hemophiliac Participants, by Human T-Lymphotropic Virus Type III/ Lymphadenopathy-Associated Virus Antibody Status and Type of Factor Used

	Factor VIII		Factor IX*	
	Antibody Positive	Antibody Negative	Antibody Positive	Antibody Negative
IgG, mg/dL†				
Median	1,800	1,375	1,590	1,660
Range	906-7,090	753-2,360	1,290-2,600	855-2,130
No.	133	48	8	7
P	<.0001		NS‡	
IgA, mg/dL§				
Median	217	196	191	176
Range	0-778	11-446	85-428	56-295
No.	132	47	8	7
P	.0054		NS	
IgM, mg/dL‖				
Median	166	134	145	116
Range	41-795	65-349	54-409	78-207
No.	132	48	8	7
P	NS		NS	
C1q binding assay¶				
Median	10	7	7	7
Range	5-33	4-22	5-11	6-11
No.	89	28	9	11
P	.0009		NS	
Staphylococcal binding assay#				
Median	83	53	37	38
Range	0-646	0-296	5-67	16-94
No.	91	28	9	11
P	.0237		NS	

*For difference between factor VIII and factor IX antibody-positive recipients: C1Q binding assay, *P*=.0142; staphylococcal binding assay, *P*=.0027; others, not significant.

†Laboratory normal range, 786 to 1,647 mg/dL.

‡NS indicates not significant.

§Laboratory normal range, 94 to 420 mg/dL.

‖Laboratory normal range, 54 to 290 mg/dL.

¶Laboratory normal range, less than 9%.

#Laboratory normal range, less than 31%.

Table 7.—Human T-Lymphotropic Virus Type III/Lymphadenopathy-Associated Virus Antigen-Specific Seroprevalence in Antibody-Positive Persons With Hemophilia, by Type of Factor Used

Antibodies*	% Positive (No. Positive/No. Tested)†	
	Factor VIII	Factor IX
p18	87 (141/162)	86 (12/14)
p25	99 (169/170)	93 (13/14)
p41	80 (131/163)	42 (5/12)‡
p18 and/or p25, but not p41	20 (32/163)	58 (7/12)

*Molecular weight of human T-lymphotropic virus/lymphadenopathy-associated virus antigen to which participant has antibody.

†Excludes 11 factor VIII recipients with trace or no recorded results concerning p18, three concerning p25, and ten concerning p41. Excludes two factor IX recipients with trace or no recorded results concerning p41.

‡*P* value for difference between factor VIII and IX recipients in rate of seropositivity to p41 antigen is .005 (Fisher's exact test).

and cytomegalovirus), our Ab− and Ab+ groups might each include (1) persons immunosuppressed by concentrates and (2) persons not affected in an immunosuppressive manner by concentrates. Second, the Ab+ group may include persons at varying time points after infection. The average incubation period for AIDS may be between two[29] and five or more years (Dale N. Lawrence, MD, unpublished data, April 1985). Similarly, at least two studies suggest that immune defects and/or clinical symptoms may become manifest in some HTLV-III/LAV-seropositive individuals without AIDS only years after seroconversion.[10,30] Additionally, Ab− persons might include (1) persons without contact with the AIDS agent and, thus, immunologically normal; (2) persons infected with, but not yet seroconverted to, HTLV-III/LAV; (3) persons infected with, but without a humoral response to, HTLV-III/LAV and thus immunologically abnormal; and (4) (theoretically), persons infected with HTLV-III/LAV, but in antigen excess in regard to the amount of antibody available for binding. Third, Ab+ persons might include (1) persons infected and immunologically damaged by HTLV-III/LAV, (2) persons able to control the infection immunologically, and (3) persons immunized, perhaps by incomplete virus in factor concentrates, and thus immunologically normal. In any of these cases, the distinction between immune test results for Ab+ and Ab− persons might be blurred. We therefore further hypothesized that any of the above possibilities might be represented by a bimodal pattern of immunologic values within the Ab− population and within the Ab+ population.

Our results suggest that both study power and the presence of mixed populations may have led to previous negative results regarding associations of HTLV-III/LAV seropositivity and immune results. First, analyses of our large number of factor VIII concentrate recipients showed a highly significant association between HTLV-III/LAV seropositivity and reduced numbers of T_H lymphocytes, as well as with reduced T_H/T_S ratios. Second, the factor IX recipients showed potentially bimodal distributions of T_H lymphocyte numbers within both the Ab+ and Ab− groups. These two serologic groups did not significantly differ immunologically; however, factor IX deficiency is a rare disorder, and our study population, although the largest yet investigated, remains small for stratified or multivariable analyses. It is therefore possible that while the Ab+ factor IX recipients with relatively higher T_H lymphocyte numbers may represent persons not yet immunologically compromised by their infection,[10,30] they may also, in part, represent persons immunized by virus that was disrupted in the production of factor IX. The Ab+ factor IX recipients with lower T_H lymphocyte numbers may represent persons infected by whole virus that survived the fractionation process. This possibility merits further epidemiologic evaluation, as well as laboratory assessment of HTLV-III/LAV antigen and cytotoxic activity in factor VIII as opposed to factor IX concentrate. While there is strong evidence that at

least some factor IX concentrates have been contaminated with infectious HTLV-III/LAV virus,[6,24] if some factor IX recipients prove immunized to HTLV-III/LAV, they would be a most important group to follow up prospectively for potential side effects and effectiveness of immunization.

Finally, two results of these analyses appear most noteworthy. First, the extent to which pooled blood product recipients can become infected with a novel infectious agent or toxin is both striking and alarming. Second, while Ab+ factor VIII recipients had depressed T_H lymphocyte numbers compared with the Ab− recipients, the medians for both Ab+ factor VIII and Ab+ factor IX recipients were within the normal range for our laboratory. This finding is unlikely to be due solely to a recency of seroconversion, since two studies suggest that seroconversion of US factor VIII concentate recipients occurred largely between late 1982 through 1983[31] or even earlier[30] and thus may permit seropositive persons an element of optimism and encourage researchers to search for cofactors of HTLV-III/LAV activation and/or the development of clinical AIDS.

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