HTLV-III/LAV Antibody and Immune Status of Household Contacts and Sexual Partners of Persons With Hemophilia

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• **We evaluated the human T-cell lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) antibody and immune status of 88 persons living with and/or sexual partners of 43 hemophiliacs, 12 of whom had AIDS, five of whom had AIDS-related complex (ARC), 17 of whom were clinically well but HTLV-III/LAV antibody positive, and nine of whom were well and HTLV-III/LAV antibody negative. No nonhemophilic household contacts (0/50) of healthy hemophiliacs were HTLV-III/LAV antibody positive; two of 33 nonhemophilic AIDS/ARC contacts were positive. One was a spouse and one a sexual partner of a hemophiliac. One of these antibody-positive contacts herself had AIDS, and one had ARC. Antibody-negative, nonhemophilic contacts of AIDS/ARC and of antibody-positive hemophiliacs had significantly lower numbers of lymphocytes, T helper lymphocytes, and T suppressor lymphocytes than did contacts of antibody-negative hemophiliacs. We conclude that risk of HTLV-III/LAV transmission may exist for spouses and/or sexual contacts of hemophiliacs with AIDS/ARC, but we cannot now determine the risk for contacts of asymptomatic hemophiliacs.**

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THE PERSON-TO-PERSON transmissibility of acquired immunodeficiency syndrome (AIDS) and the "transmissibility" of immune defects possibly related to that agent have been of concern to the medical community. A sexual pattern of AIDS transmission has been postulated on the basis of the prevalence of AIDS in persons at risk for sexually transmitted diseases. Further evidence for sexual transmission of AIDS includes a clustering of cases among a group of homosexual partners[1] and individual case reports of AIDS or immune dysfunction in sexual partners of persons with AIDS or at risk for AIDS.[2-4] Parent-to-child transmission has also been postulated, with the strongest evidence supporting possible perinatal transmission.[5-8] It would thus appear that in certain cases sexual or parent-child contact may permit the transmission of AIDS.

Persons with hemophilia are at risk for AIDS.[9] Studies of the immune function of hemophilic patients' sexual partners have shown results that are equivocal[10,11] or normal.[12] One study of the immune function of siblings and spouses of hemophiliacs found that these persons' immune studies were normal.[13] The relationship of these findings to AIDS is somewhat unclear, however, because the studies did not determine the antibody or virus status of these individuals to human T-cell lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV), now determined to be the cause of AIDS.[14-18] Hemophiliacs with immune abnormalities may not necessarily be infected with HTLV-III/LAV, since factor concentrate itself may be immunosuppressive, even when produced from a population of donors not at risk for AIDS.[19-21] However, case reports of lymphadenopathy or AIDS in sexual partners of hemophiliacs suggest that AIDS transmission may be possible by persons within this AIDS risk group.[22,23]

We have found that three fourths of US hemophilia A patients have antibodies to HTLV-III/LAV and that HTLV-III/LAV serologic status shows significant correlations with laboratory immune status findings.[24] This does not necessarily mean, however, that these individuals are infectious for this virus. In this study, we evaluate the status of antibodies to HTLV-III/LAV of persons having close contact with hemophiliacs without symptoms of AIDS, with AIDS, or with potentially AIDS-related symptoms (AIDS-related complex [ARC]). Furthermore, because seronegativity for antibody to HTLV-III/LAV does not necessarily preclude infection with that agent,[25] we also examined these persons' immune function.

SUBJECTS AND METHODS

Households of Hemophiliacs Without Symptoms of AIDS

All households voluntarily enrolled in 1983 to 1984. Participants included nine households from the New York City area that consisted of seven spouses and/or sexual partners and two parents of nine index hemophiliacs, without clinical symptoms of AIDS, and 16 households from Georgia that consisted of one spouse and/or sexual partner, 17 siblings, 24 parents, and one grandparent of 16 index hemophilic participants. Two of these index hemophiliacs had hemophilia B; four of the siblings themselves had hemophilia A. One household was enrolled from Ohio, including an 11-year-old hemophilic participant, his mother, and his brother. Median age of these household partici-

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pants was 31.5 years, with a range of 1.5 to 83 years.

AIDS/ARC

Household members, spouses, and/or sexual partners of 12 hemophilic AIDS patients reported to the Centers for Disease Control, Atlanta, voluntarily enrolled, including nine spouses and/or sexual partners, four siblings, four parents, and nine children. Five spouses and/or sexual partners, one son, and two parents of five hemophilic persons with ARC were enrolled. Three of the index hemophiliacs had lymphadenopathy, one had chronic *Isospora belli* enteritis and weight loss, and one had retinal exudates and fever of unknown origin.

Methods

Serum specimens were tested for antibody to HTLV-III/LAV by Western blot analysis. The HTLV-III/LAV was separated by ultracentrifugation of culture supernates from HTLV-III/LAV-infected, phytohemagglutinin-stimulated human lymphocytes[17] through a 30% wt/wt sucrose cushion (80,000 g for one hour). The pellets were dissolved in 0.01M TRIS, pH 8.0, containing 1% sodium dodecyl sulfate, 0.25 mg/mL of bromophenol blue, 10% glycerol, and 5% 2-mercaptoethanol, and were heated at 65 °C for 30 minutes. Western blot analyses were performed by the method of Tsang et al.[28] Serum specimens were tested at a 1:100 dilution, and banding patterns were compared to a known positive control serum. Serologic reactions with any combination of the 18kd, 25kd, and 41kd proteins of HTLV-III/LAV were scored as positive.

Lymphocyte subpopulations were quantitated by indirect immunofluorescence on a fluorescence-activated cell sorter (FACS IV, Becton-Dickinson, Sunnyvale, Calif), using commercial monoclonal antibodies (OKT3 for T cells, OKT4 for T helper/inducer cells [T_H], and OKT8 for T suppressor/cytotoxic cells [T_S], Ortho Diagnostics, Raritan, NJ) and fluorescein-conjugated anti-mouse immunoglobulin (Centers for Disease Control).[27,28] Immunoglobulins G, A, and M were quantitated for 32 participants, by nephelometry (Baker Chemicals, Allentown, Pa).

Serum specimens from some participants were tested for antibodies to cytomegalovirus by complement fixation (CF) (n=30) and/or indirect hemagglutination (IHA) (n=45)[29]; herpes simplex virus types 1 and 2 by CF (n=30) and/or IHA (n=41)[30]; and Epstein-Barr virus viral capsid antigen, nuclear antigen, and early antigen (n=65).[31] Tests for hepatitis B virus surface antigen (HBsAg), antibody to hepatitis B virus surface antigen (HBsAb), and antibody to the hepatitis B core antigen (HBcAb) were done by radioimmunoassay (n=30) (Abbott Laboratories, North Chicago, Ill).

Table 1.—HTLV-III/LAV Antibodies and T-Cell Subsets in Hemophilic Sibships*

Sibship	Hemophiliac	Antibodies to	T_H†	T_S†	T_H/T_S†
A	1‡	18, 41	ND	ND	ND
	2	18, 25, 41	ND	ND	ND
B	3	18, 25	795	543	1.5
	4	18, 25	1,341	711	1.9
	5	18, 25, 41	840	1,320	0.6
C	6	18, 25, 41	959	679	1.4
	7	18, 25, 41	548	1,158	0.5
D	8	18, 25, 41	631	707	0.9
	9	18, 25, 41	314	470	0.7
Laboratory normal ranges					
. . .	. . .	. . .	408-1,583	190-820	1.0-3.9

*HTLV-III/LAV indicates human T-cell lymphotropic virus type III/lymphadenopathy-associated virus; T_H, T helper/inducer cells; T_S, T suppressor/cytotoxic cells; and ND, not done.
†T-cell subsets given as cells per cubic millimeter.
‡Individual 1 had AIDS at the time of testing.

Table 2.—HTLV-III/LAV Antibodies and T-Cell Subsets in Antibody-Positive, Nonhemophilic Participants*

Subject	Antibodies *to*	T_H†	T_S†	T_H/T_S†	Clinical Status of Nonhemophiliac*
10	18, 41	299	581	0.5	AIDS
11	18, 25, 41	384	561	0.7	ARC

*HTLV-III-/LAV indicates human T-cell lymphotropic virus type III/lymphadenopathy-associated virus; T_H, T helper/inducer cells; T_S, T suppressor/cytotoxic cells; AIDS, acquired immunodeficiency syndrome; and ARC, AIDS-related complex.
†T-cell subsets given as cells per cubic millimeter.

Statistical Analysis

Demographic, immunologic, and serologic results were compared between various groups using the Wilcoxon rank-sum test[32] or Fisher's exact test (FET). Significance level for all statistical analyses was .05.

RESULTS

Six hemophilia-AIDS patients were tested for HTLV-III/LAV antibody; five had positive results. Four ARC patients were tested; all had positive results. Seventeen index hemophilia A patients without symptoms of AIDS had antibodies to HTLV-III/LAV; seven hemophilia A and the two hemophilia B patients did not have HTLV-III/LAV antibodies. In all four households with more than one person with hemophilia, all hemophilic members were positive for HTLV-III/LAV antibodies. The antibody patterns were extremely similar, but not necessarily identical, among siblings (Table 1). No consistent T-cell subset pattern was evident within any of these sibships. All hemophilic members of sibships B, C, and D were positive for HBcAb and HBsAb but negative for HBsAg.

One spouse of an AIDS patient and one sexual partner (non-household member) of an ARC patient were positive for antibody to HTLV-III/LAV (Table 2). The rate of seropositivity was therefore 6% (2/33) for nonhemophilic individuals in AIDS/ARC households, with 12% (2/17) of AIDS/ARC households and/or sexual partner clusters having a nonhemophilic HTLV-III/LAV-antibody-positive member. This rate of seroprevalence did not differ significantly from that for household members of healthy, HTLV-III/LAV seropositive hemophiliacs (0/29) or for household contacts of all healthy hemophiliacs (0/50). The seroprevalence rates for spouses and/or sexual partners of AIDS/ARC patients was 14% (2/14) and for spouses and/or sexual partners of all healthy hemophiliacs was 0 (0/8). Similarly, the proportion of AIDS/ARC households with a seropositive nonhemophiliac did not differ significantly from that of households of healthy, HTLV-III/LAV-seropositive hemophiliacs (0/17) or for households of all healthy hemophiliacs (0/26).

One of these nonhemophilic, seropositive persons herself had AIDS at the time of testing. This person's hemophilic spouse was HTLV-III/LAV antibody negative in testing done after AIDS was diagnosed. The couple was not sexually active in the 1½ years prior to diagnosis and had had vaginal intercourse approximately twice a year prior to that time. The wife did not assist the patient in administering his factor concentrate.

Table 3.—Lymphocyte Populations of Nonhemophilic, HTLV-III/LAV-Antibody-Negative Participants, by Status of Index Hemophiliacs*

	AIDS/ARC (n=15)†	Ab+‡ (n=28)	Ab−‡ (n=13)
Total lymphocyte count, cells/cu mm			
Median	2,059	2,188	3,060
Range	300-3,234	55-4,080	1,710-5,841
T_H count, cells/cu mm			
Median	662	884	1,310
Range	129-1,876	32-1,510	701-2,317
T_S count, cells/cu mm			
Median	377	612	734
Range	51-781	8-1,320	462-1,190

*HTLV-III/LAV indicates human T-cell lymphotropic virus type III/lymphadenopathy-associated virus; T_H, T helper/inducer cells; and T_S, T suppressor/cytotoxic cells.

†AIDS/ARC (acquired immunodeficiency syndrome/AIDS-related complex) nonhemophilic participant n=16 for lymphocyte count.

‡Asymptomatic, HTLV-III/LAV-antibody-positive index hemophiliac (Ab+); asymptomatic, HTLV-III/LAV-antibody-negative index hemophiliac (Ab−).

Table 4.—Lymphocyte Populations of Asymptomatic Hemophilic Participants, by Their HTLV-III/LAV Antibody Status*

	Ab+ (n=20)	Ab− (n=6)†	P Value‡
Total lymphocyte count, cells/cu mm			
Median	2,223	3,657	.0080
Range	690-3,450	2,244-9,546	. . .
T_H count, cells/cu mm			
Median	692	1,772	.0068
Range	179-1,341	785-3,150	. . .
T_S count, cells/cu mm			
Median	709	1,299	NS
Range	261-1,587	494-1,643	. . .

*HTLV-III/LAV indicates human T-cell lymphotropic virus type III/lymphadenopathy-associated virus; Ab+, asymptomatic, HTLV-III/LAV-antibody-positive index hemophiliac; Ab−, asymptomatic, HTLV-III/LAV-antibody negative hemophiliac; T_H, T helper/inducer cells; T_S, T suppressor/cytotoxic cells.

†For antibody-negative participants, n=7 for lymphocyte count.

‡*P* value for difference between Ab+ and Ab−: NS.

The second antibody-positive individual had lymphadenopathy, as did her sexual partner, at the time of testing. The couple engaged in rectal as well as vaginal intercourse, and the female partner assisted the hemophiliac in administering his factor concentrate. The hemophiliac was himself positive for antibody to HTLV-III/LAV p18 and p41 antigens; he had 540 T_H cells/cu mm, 450 T_S cells/cu mm, and a T_H/T_S ratio of 1.2. A second sexual partner of this index hemophiliac was negative for antibodies to HTLV-III/LAV and was immunologically normal. Her last intercourse with the index participant had been 1½ years before the development of his lymphadenopathy.

We last evaluated whether immunologic differences existed for households with known exposure to an AIDS or an HTLV-III/LAV-antibody-positive (Ab+) individual, despite the household members' currently negative HTLV-III/LAV antibody status. The following HTLV-III/LAV antibody-negative, nonhemophilic participants were therefore compared: AIDS/ARC households (AIDS/ARC HH), households of Ab+ hemophilia A participants (Ab+ HH), and households of antibody-negative hemophilia A participants (Ab− HH). These groups did not differ significantly in age, sex, race, or other viral serologic findings. They did differ, however, in their lymphocyte numbers (Table 3). Numbers of T_S cells were lower in AIDS/ARC HH than in Ab+ HH (P=.038), but total lymphocyte and T_H numbers were similar for these two groups. Lymphocytes, T_H, and T_S numbers were significantly lower for AIDS/ARC HH than for Ab− HH (P=.003, P=.014, and P=.0005, respectively). Similarly, these counts were lower for Ab+ HH than for Ab− HH (P=.003, P=.016, and P=.044, respectively). These differences were not due to a few households nor to households from one geographic area, and reflected similar, but more extreme, differences in lymphocyte and T_H numbers between the Ab+ and Ab− hemophiliacs themselves (Table 4). Within each group (AIDS/ARC HH, Ab+ HH, and Ab− HH), no immunologic or non-HTLV-III/LAV serologic differences were found between those who did or did not assist the hemophilic participants in administering factor. Furthermore, within each group, immunologic findings did not differ between persons having sexual or nonsexual relations with the index hemophiliacs. (HBsAb was present in high titers in two spouses and/or sexual partners of ARC hemophiliacs.)

COMMENT

Persons with hemophilia generally have stable home environments. Thus, ever since they were recognized as being at heightened risk of AIDS,[9] there has been concern and speculation concerning AIDS transmissibility to long-term sexual partners and household members of this at-risk population. Transmission patterns have appeared similar to those of hepatitis B virus, a virus for which this AIDS-risk group is also at risk.[33] Past evaluations of immune parameters in sexual partners and/or siblings of hemophiliacs have suggested that risk is low.[10-13] However, the number of hemophilic contacts evaluated was small and it was not possible to differentiate households of hemophiliacs exposed to the AIDS agent from those not exposed to the agent. Furthermore, evidence from other at-risk populations supports the possibility of sexual transmission of AIDS[1-3] and of HTLV-III/LAV.[34] Since a large proportion of hemophilic patients receiving factor concentrate therapy appear to have had contact with HTLV-III/LAV, we thought it imperative to evaluate both sexual and nonsexual contacts of hemophiliacs with and without symptoms of AIDS.

Only two (2.3%) of 88 individuals without hemophilia had antibody to HTLV-III/LAV at the time of this evaluation. Both individuals had contact with a hemophiliac with AIDS or ARC; thus, the rate of positivity was two of 33 for nonhemophiliac AIDS/ARC household members and two of 14 for spouses and/or sexual partners of AIDS/ARC patients, as opposed to zero of 29 household members (including seven spouses) of hemophiliacs who were HTLV-III/LAV antibody positive but were clinically well. This difference is not statistically significant; further household evaluations will be needed to determine if it occurred by chance alone or if it might reflect differential infectivity (eg, higher viral titers in saliva or semen) in symptomatic-presymptomatic, as opposed to asymptomatic, persons. We did not find HTLV-III/LAV seropositivity limited to persons having ongoing

sexual contact with, or administering factor concentrate to, the index hemophiliac.

The time between HTLV-III/LAV infection and seroconversion is currently unknown. We therefore compared seronegative nonhemophilic household members and/or sexual partners grouped by their index hemophilia A patient, as follows: AIDS/ARC, Ab+, and Ab. Signs of immune dysfunction might be indicative of early infection. The Ab− HH were chosen as the best comparison group since theoretically their other exposures and risks would be similar to those of the first two groups. These groups did not differ in age, sex, or race. Findings of immune studies in all three groups were within the normal range for our laboratory, but AIDS/ARC HH and Ab+ HH had significantly lower numbers of both T_H and T_S lymphocytes than did Ab− HH. This difference was even more pronounced for the hemophiliacs in these households. While this could reflect an early response to HTLV-III/LAV infection, we think these results should be interpreted with great caution pending prospective serial evaluation of these households and similar evaluations of other hemophilic households. However, they could be epidemiologically meaningful, in light of a recent report suggesting that some antibody-negative sexual partners of hemophiliacs were themselves virus positive on repeated cultures.[35]

Serologic results in two groups of US hemophiliacs suggest HTLV-III/LAV seroconversion became widespread between 1981 and 1983.[36] It is therefore encouraging to find a low rate of HTLV-III/LAV seroprevalence, as well as normal immune function, in household contacts of hemophiliacs. Continued evaluation of this population will provide important information concerning transmissibility of HTLV-III/LAV and its relation to clinical AIDS, and will permit us to quantify and define the real, albeit apparently small, risk of HTLV-III/LAV infection in close contacts of hemophiliacs.

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