

AIDS Retrovirus Antibodies in Hemophiliacs Treated With Factor VIII or Factor IX Concentrates, Cryoprecipitate, or Fresh Frozen Plasma: Prevalence, Seroconversion Rate, and Clinical Correlations

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Antibodies to the AIDS retrovirus, specifically to human T cell lymphotropic virus, type III, and AIDS-associated retrovirus, were detected with increasing prevalence in a population of 190 hemophiliacs from western Pennsylvania between 1981 and 1984: 7.7% in 1981, 20.0% in 1982, 45.5% in 1983, and 62.5% in 1984. The seropositive included approximately three fourths of those receiving factor VIII concentrate, nearly one third of those receiving factor IX concentrate, nearly one fifth of those receiving cryoprecipitate, and none of those receiving fresh frozen plasma. The seroconversion rate, determined on 43 seropositive hemophiliacs from this group who were serially sampled, was 0% in 1977, 4.7% in 1978, 4.9% in 1979, 2.6%

in 1980, 10.5% in 1981, 52.9% in 1982, 87.5% in 1983, and 100% in 1984. Of 27 seropositive for three or more years (since 1982 or before), four (15%) have developed AIDS and seven (26%), diffuse lymphadenopathy (ARC); of 16 seropositive for less than three years, none has developed AIDS and three (19%) have developed ARC. The mean time from seroconversion to onset of ARC, 0.8 ± 0.2 years (SEM), was shorter ($P < .001$) than the time to onset of AIDS, 4.1 ± 0.6 years. These findings confirm the widespread presence of AIDS retrovirus and support the association of these retroviruses with the acquired immunodeficiency syndrome and related conditions.

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THREE GROUPS of investigators have isolated retroviruses from patients with the acquired immunodeficiency syndrome (AIDS) and AIDS-related syndromes. Termed lymphadenopathy-associated virus (LAV),¹ human T cell lymphotropic virus, type III (HTLV-III),² and AIDS-associated retrovirus (ARV),³ these viruses appear to be nearly identical, with similar morphological, cytopathic, and molecular features.¹⁻⁵

In hemophilia-related AIDS, transmission of the AIDS agent presumably occurs through exposure to blood products. Most hemophiliacs who have developed AIDS have been treated with factor VIII concentrate,⁶ although the disease has been detected in several factor IX concentrate-treated hemophiliacs.⁶ In addition, although AIDS has not yet occurred in hemophiliacs who have been treated with cryoprecipitate, two patients with von Willebrand's disease who were treated with cryoprecipitate have developed AIDS.⁷ Correspondingly, AIDS retrovirus seropositivity has been detected primarily in those hemophiliacs receiving factor VIII concentrate,⁸⁻¹² to a lesser degree in those receiving factor IX concentrate, and in few, if any, receiving cryoprecipitate.⁸⁻¹²

This study evaluated the entire population of hemophiliacs from western Pennsylvania, exposed to factor VIII concen-

trate, factor IX concentrate, cryoprecipitate, or fresh frozen plasma, for the presence of antibodies to AIDS retroviruses, specifically antibodies to HTLV-III and ARV, between 1981 and 1984. This period coincided with the occurrence of AIDS in patients with hemophilia. Tests for antibodies to AIDS retrovirus isolates were used to determine whether any significant difference in antibody prevalence would be observed.

MATERIALS AND METHODS

Clinical population. Of the 190 hemophiliacs who constituted the entire hemophilic population in western Pennsylvania, 155 had hemophilia A and 35 had hemophilia B. Those with hemophilia A included 82 treated with factor VIII concentrate, 59 with cryoprecipitate, and 14 with factor IX concentrate because of the presence of an inhibitor to factor VIII. The last exposure to factor VIII concentrate in the latter group was before early 1976. Those with hemophilia B included 16 treated with factor IX concentrate and 19 with fresh frozen plasma. The mean age for those receiving factor VIII concentrate was 29.6 years, for factor IX concentrate 26.8 years, for cryoprecipitate 18.7 years, and for fresh frozen plasma 24.8 years.

Serum samples. Citrated plasma samples, obtained from all 190 hemophiliacs during routine clinical evaluation at the Hemophilia Center of Western Pennsylvania, were available for the assays described below. Only one blood sample per hemophiliac was tested in this study, specifically the first sample available beginning in 1984 and going back to 1981. In addition, 43 of the seropositive hemophiliacs on whom serial samples were available annually or biannually between 1977 and 1984 constituted the population base for determining the AIDS retrovirus seroconversion rate. This group, selected solely on the basis of their seropositivity and availability of serial samples, did not differ from the original 190 hemophiliacs in age, race, or blood product usage. Samples were frozen at -20°C for several months to 6 years before the study.

Viral antibody tests. Antibodies (IgG) to HTLV-III were measured by an enzyme-linked immunosorbent assay (ELISA) technique (Electronucleonics, Inc, Columbia, Md), using an inactivated HTLV-III antigen from a previously established cell line.¹¹ Briefly, a 1:100 dilution of test plasma was added to a microassay-plate well that had been coated with inactivated virus antigen. After a 30-minute, 37°C incubation, the unbound material was washed and aspirated. Goat antiserum to human IgG, both heavy and light chain

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specific, labeled with horseradish peroxidase, was then added and incubated for 30 minutes at 37 °C. Unbound material was washed and aspirated, and a chromogen (*o*-phenylenediamine dichloride, or OPD) was added. After a 10-minute, room temperature incubation, the reaction was stopped with 2N sulfuric acid, and the absorbance was read spectrophotometrically at 492 nm. Samples giving an optical density >0.100 in two separate assays were considered positive.

For seroconversion data only, antibodies to HTLV-III (IgG) were detected by an ELISA technique (Abbott Laboratories, Chicago), using beads coated with inactivated HTLV-III antigen from the Hg/HTLV-III B cell line. A 1:400 dilution of test plasma was added to each bead-containing microassay-plate well and was incubated 65 minutes at 40 °C. Unbound material was washed and aspirated. Goat antihuman IgG, conjugated to horseradish peroxidase, was added and incubated for 130 minutes at 40 °C. Unbound material was washed and aspirated, and the chromogen (OPD) was added and incubated for 30 minutes at room temperature. The reaction was stopped with 1N sulfuric acid, and absorbance was read at 492 nm. Samples giving an optical density greater than or equal to a cutoff value, determined daily by negative and positive controls, were considered positive.

Antibodies to ARV were measured, as previously described,³ by indirect immunofluorescence (IFA), using goat antiserum to human IgG, an adult human T cell line, HUT-78, infected with ARV-2, and a 1:10 dilution of test plasma. Four of the original 190 samples were of insufficient quantity for ARV antibody testing. Samples that were HTLV-III (Electronucleonics) seropositive, ARV seronegative or HTLV-III (Electronucleonics) seronegative, ARV seropositive were retested at a 1:5 dilution of test plasma, and the latter result was used in this study. There was no significant difference in the results obtained by ELISA with the HTLV-III antigen and those obtained by IFA with the ARV-2 antigen.

For the Western blot procedures (Electronucleonics), purified HTLV-III virus was reduced and electrophoretically resolved by polyacrylamide gel electrophoresis, using 12.5% running and 4% stacking gels at 150 V, pH 8.8. The resolved protein was transferred to nitrocellulose electrophoretically at 36 V for four hours, blocked

with 20% goat serum in phosphate-buffered saline (PBS)/0.05% Tween (J.T. Baker Chemical Co, Phillipsburg, NJ), and cut into 0.5-cm strips. These strips were loaded with a 1:200 dilution of test plasma, incubated for two hours at room temperature, washed, and then incubated with a 1:2,000 dilution of goat antihuman IgG (heavy and light chain) conjugated to horseradish peroxidase. After serial washings, the substrate chloro-naphthol was added, and the strips were developed, blotted on bibulous paper, and dried. The test was considered positive if either the viral p24 or gp41 proteins were present.

Statistical analysis was performed by Student's *t* test.

RESULTS

The progressive yearly increase in AIDS retrovirus seropositivity in this group of hemophiliacs between 1981 and 1984 is shown in Table 1, with an overall prevalence for these years of 42.1% (80/190), respectively. The 80 seropositive included 74.4% of those receiving factor VIII concentrate, 30.0% of those receiving factor IX concentrate, 17.0% of those receiving cryoprecipitate, and 0% of those receiving fresh frozen plasma, respectively. The prevalence of seropositivity in factor IX concentrate-treated patients with hemophilia B was similar to that of factor IX concentrate-treated patients with hemophilia A and an inhibitor: of nine seropositive, five had hemophilia B and four had hemophilia A with an inhibitor.

Forty-three seropositive hemophiliacs from the group, on whom serially samples were available between 1977 and 1984, were further studied. None were seropositive in 1977 and two were seropositive in 1978. An increase in seroconversion rate occurred after 1980, with the most marked increase appearing in 1982 (Table 2). Seropositivity persisted in all individuals on repeat testing at later dates, and none reverted back to seronegative after becoming seropositive. When the date of first seroconversion was looked at in each blood product treatment group (Table 3), the first of 25 factor VIII concentrate-treated hemophiliacs seroconverted in 1978, the first of eight factor IX concentrate-treated hemophiliacs seroconverted in 1978, and the first of ten cryoprecipitate-treated hemophiliacs seroconverted in 1981. (The latter is a locally prepared single-donor product.) The eight factor IX concentrate-treated hemophiliacs included four factor IX-deficient individuals, three of whom seroconverted in 1983

Table 1. Prevalence of AIDS Retrovirus Antibodies in a Population of 190 Hemophiliacs

	AIDS Retrovirus Antibody	
	Number	Percentage
Prevalence by year sampled		
1981	2/26 (2/26)	7.7 (7.7)
1982	8/40 (6/40)	20.0 (15.0)
1983	20/44 (16/42)	45.5 (38.1)
1984	50/80 (54/78)	62.5 (69.2)
Total	80/190 (78/186)	42.1 (41.9)
Prevalence by blood product exposure		
F VIII	61/82 (60/81)	74.4 (74.1)
F IX	9/30 (10/30)	30.0 (33.3)
Cryo	10/59 (8/57)	17.0 (14.0)
FFP	0/19 (0/18)	0 (0.0)
Total	80/190 (78/186)	42.1 (41.9)

The data presented is the number (or percentage) seropositive over the total number tested for HTLV-III antibody, with similar data based on ARV antibody in parentheses. Only one blood sample per hemophiliac was used in this study—the first sample available from 1984 back to 1981. F VIII, factor VIII concentrate; F IX, factor IX concentrate; CRYO, cryoprecipitate; FFP, fresh frozen plasma.

Table 2. AIDS Retrovirus Seroconversion Rate

Year	No. Becoming Seropositive/ No. Remaining Seronegative	Percentage
1977	0/43	0.0
1978	2/43	4.7
1979	2/41	4.9
1980	1/39	2.6
1981	4/38	10.5
1982	18/34	52.9
1983	14/16	87.5
1984	2/2	100.0

The determination of seroconversion rate was based on serial samples on 43 AIDS retrovirus seropositive hemophiliacs between 1977 and 1984. Seroconversion rate was expressed as a percentage and defined for each year as the number becoming seropositive (seroconverting) in a given year over the number remaining seronegative that year.

Table 3. AIDS Retrovirus Seroconversion in 43 Seropositive Hemophiliacs

	1977	1978	1979	1980	1981	1982	1983	1984
F VIII*								
1†	-	-	-	-	+	+		+
2†	-	-	-	+	+	+	+	+
3†	-	+	+	+	+	+		+
4†	-	-	-	-	-	+	+	+
5‡	-	-	+	+	+		+	+
6‡	-	-	-	-	-	+		+
7‡	-	-	-	-	-	+		+
8‡	-	-	-	-	+	+		+
9‡	-	-	-	-	-	+		+
10‡	-	-	-	-	-	+		+
11‡	-	-	-	-	-	-	+	+
12‡	-	-	+	+	+	+		+
13	-	-	-	-	-	+	+	+
14	-	-	-	-	-	+	+	+
15	-	-	-	-	-	+	+	+
16	-	-	-	-	-	-	+	+
17	-	-	-	-	-	-	+	+
18	-	-	-	-	+	+	+	+
19	-	-	-	-	-	+		+
20	-	-	-	-	-	+	+	+
21	-	-	-	-	-	+	+	+
22	-	-	-	-	-	-	+	+
23	-	-	-	-	-	+	+	+
24	-	-	-	-	-	+	+	+
25	-	-	-	-	-	-	+	+
F IX*								
1	-	-	-	-	-	-	+	+
2	-	-	-	-	-	-	-	+
3‡	-	-	-	-	-	-	+	+
4	-	-	-	-	-	-	+	+
5	-	-	-	-	-	+	+	+
6	-	+	+	+	-	+		+
7	-	-	-	-	-	-	+	+
8‡	-	-	-	-	-	-	+	+
Cryo*								
1	-	-	-	-	-	-		+
2	-	-	-	-	-	-	+	+
3	-	-	-	-	-	+		+
4	-	-	-	-	-	-	+	+
5	-	-	-	-	-	+	+	+
6	-	-	-	-	-	-	+	+
7	-	-	-	-	-	-	+	+
8	-	-	-	-	-	+		+
9	-	-	-	-	-	+		+
10	-	-	-	-	+			

*F VIII refers to hemophiliacs treated with factor VIII concentrate, F IX refers to those treated with factor IX concentrate, and cryo refers to those treated with cryoprecipitate.

†Patient developed AIDS.

‡Patient developed diffuse lymphadenopathy (ARC).

and the fourth of whom seroconverted in 1984; the other four factor IX concentrate-treated hemophiliacs included four factor VIII-deficient individuals with an inhibitor to factor VIII whose last exposure to factor VIII was before early 1976, one of whom seroconverted in 1978, one in 1982, and two in 1983.

From this group of 43 sequentially sampled hemophiliacs, four (9%) developed AIDS and ten (23%) developed diffuse

lymphadenopathy (ARC). Of the 27 who have been seropositive for three or more years (1982 or earlier seroconversion), four (15%) have developed AIDS and seven (20%) have developed diffuse lymphadenopathy (Table 3). In addition, one (4%) developed immune thrombocytopenia (also had AIDS). Of the 16 who have been seropositive for less than three years (1983 or later seroconversion), none has developed AIDS and three (19%) have developed ARC. The mean time from seroconversion to onset of ARC in all ten with ARC was 0.8 ± 0.2 years (SEM) (range, 0 to 2) which was significantly shorter ($P < .001$) than the time to onset of AIDS in all four with AIDS, 4.0 ± 0.6 years (range, 3 to 6).

The total yearly blood product usage (units per year) in patient sampled in 1984 (Table 4) revealed that a significantly greater amount of factor VIII concentrate was used in seropositive hemophiliacs than in seronegative hemophiliacs. The factor IX concentrate usage appeared to be twice as high in seropositive hemophiliacs as in seronegative hemophiliacs, but this difference was not significant. There appeared to be no difference in cryoprecipitate usage between seropositive and seronegative hemophiliacs. Because of small numbers and incomplete data, comparisons could not be made for 1981 through 1983, except for factor VIII concentrate usage in 1983, which was significantly greater ($P < .005$) (data not shown) in seropositive hemophiliacs compared with seronegative hemophiliacs.

DISCUSSION

The increasing prevalence of antibodies to AIDS retrovirus in this group of hemophiliacs between 1981 and 1984 parallels the occurrence of AIDS in the hemophiliac population.⁶ Further, the strong correlation between the presence of antibodies to HTLV-III and ARV in these patients measured by different assays is consistent with data showing that HTLV-III and ARV (as well as LAV) are variants of the same virus.^{4,5} The fact that all hemophiliacs with AIDS or AIDS-related illness in this study were AIDS retrovirus (both HTLV-III and ARV) seropositive supports the close association of these retroviruses with AIDS and AIDS-related conditions. Detection of AIDS retrovirus antibodies (HTLV-III or ARV) in the majority of western Pennsylva-

Table 4. Blood Product Usage and AIDS Retrovirus Antibody Status in 1984

Blood Product	Antibody (+)	Antibody (-)	P
F VIII concentrate	66,637 ± 13,086 (n = 41)	18,603 ± 7,354 (n = 6)	<.005
F IX concentrate	58,053 ± 12,951 (n = 4)	27,310 ± 7,101 (n = 5)	NS
Cryoprecipitate	19,000 ± 6,223 (n = 3)	19,925 ± 5,476 (n = 8)	NS
Fresh frozen plasma	— (n = 0)	7,585 ± 4,819 (n = 2)	—

Data are based on blood product usage between September 1983 and August 1984. Figures in parentheses are the number on whom data were available. Data presented are based on HTLV-III antibody positive and negative hemophiliacs; corresponding data for ARV antibody positive and negative were not significantly different. Results are expressed as the mean ± standard error (SEM).

nia hemophiliacs, similar to data reported in San Francisco homosexuals³ and hemophiliacs¹² and in Australian bisexual and homosexual males,¹³ demonstrates that exposure to the AIDS retrovirus is widespread geographically. Moreover, the AIDS virus appears to have been present here at least since 1978, one year earlier than that found in a smaller study¹⁴ by Eyster et al, but still consistent with the occurrence of the earliest cases of AIDS in the United States in 1978 and 1979.

The predominance of AIDS retrovirus seropositivity in factor VIII concentrate-treated hemophiliacs parallels the higher incidence of AIDS in hemophiliacs treated with this blood product. The markedly lower prevalence of antibodies in hemophiliacs exposed to factor IX concentrate is consistent with the infrequent occurrence of AIDS in this group.⁶ The presence of such antibodies in cryoprecipitate-treated hemophiliacs, a group in which AIDS has not yet occurred, further supports the recommendation of the use of heat-treated factor VIII concentrate to prevent transmission of potentially infectious retroviruses.^{15,16}

The marked increase in seroconversion that occurred in 1982 is in agreement with a similar study of hemophiliacs

treated only with factor VIII concentrate.¹⁴ The time of occurrence and the percentage of virus-exposed individuals developing disease are a noteworthy observation. They offer some indication of the risk of pathogenic consequences after exposure to the virus in hemophiliacs and mirror similar observations with other AIDS risk groups.¹⁷ Further, these data suggest that a large number of hemophiliacs were exposed through their clotting factor treatment to infectious viruses and not just viral antigens. The short time between the date of seroconversion and the development of diffuse adenopathy in ten hemophiliacs supports the view that this clinical sign can be an early manifestation of AIDS retrovirus infection or exposure. The reason for the longer time between seroconversion and development of AIDS in four hemophiliacs is not clear but probably reflects the indolent disease course that eventually leads to widespread compromise of the immune system by AIDS retrovirus infection.

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