A cohort of 181 patients with hemophilia A (149) and hemophilia B (32) cared for at the Hemophilia Center of Western Pennsylvania was followed to determine human immunodeficiency virus (HIV) seroprevalence, seroconversion rate, and clinical and immunologic correlates of HIV infection. By December 1986, 82 (45%) were HIV seropositive, and of these, ten (12%) had developed AIDS. 28 (34%) had symptomatic HIV infection (CD4 cell class III, IV), of whom 14 (17%) had AIDS-related complex (ARC), and 44 (54%) had asymptomatic HIV infection (CD4 cell class II). The HIV seropositive group included 82% of those treated with factor VIII concentrate (97% severe, 3% moderate), 48% of those treated with factor IX concentrate (92% severe, 8% moderate), 10% of those treated with cryoprecipitate (67% severe, 33% moderate), and none of those treated with fresh frozen plasma. Based on 77 serially sampled HIV seropositive hemophiliacs (1977 to 1986), peak seroconversion occurred in 1982, with 14% (11 of 77) occurring since 1984. With increasing time from seroconversion, both T4 lymphocyte number and function (the latter measured by growth in soft agar [T colony assay]) progressively declined: T4 number declined to 135 ± 26/mm3 (SEM), and colony count declined 1193 ± 537 (control 3851 ± 387) by 5 years after seroconversion. In those developing AIDS, total T4 fell below 100/mm3 (33 ± 8/mm3) at diagnosis. In this cohort, the overall AIDS incidence is 5.5% (12% among the HIV seropositive) and in those seropositive 5 or more years, the AIDS incidence approaches 32%.

Although fewer than 1% of the total number of AIDS cases occur in hemophiliacs, the incidence of AIDS in this group is quite high (greater than one case per 100). Moreover, AIDS has become a leading cause of death in this group.

Hemophiliacs differ from other high risk groups not only in the rarity of sexually transmissible diseases and intravenous (IV) drug use but also in the expected cessation of further human immunodeficiency virus (HIV) exposure because of current HIV antibody donor screening and heat inactivation of blood products. Although a recent Centers for Disease Control (CDC) survey has shown a decline in the rate of AIDS cases in hemophiliacs since mid-1985, the long-term prognosis of HIV-infected hemophiliacs is not yet known, since the peak in seroconversion in hemophiliacs occurred in 1982, and the interval from infection to development of AIDS may be 5 years or longer.

Previous study in this laboratory has revealed that hemophiliacs with AIDS-related illness show impaired cellular immunity, specifically, depressed lymphocyte function as measured by in vitro T lymphocyte growth in soft agar (T colony assay). Although immune parameters that predict the development of AIDS are unknown, a recent study has shown that a significant decrease in T4 helper lymphocytes in HIV antibody-positive homosexuals, drug users, and hemophiliacs is associated with an increased risk for the development of AIDS.

In this study, we report the seroprevalence, seroconversion, AIDS incidence, and immunologic correlates of HIV infection in a cohort of 182 hemophiliacs from western Pennsylvania.

**MATERIALS AND METHODS**

**Clinical data.** The cohort studied here has been previously reported in detail elsewhere. One hundred fifty had classic hemophilia or hemophilia A (congenital factor VIII:C deficiency), 79 of whom were treated with factor VIII concentrate, 57 with cryoprecipitate, and 14 (who had inhibitors to factor VIII) with factor IX concentrate. The remaining 31 had Christmas disease or hemophilia B (congenital factor IX:C deficiency), 14 of whom were treated with factor IX concentrate and 17 with fresh frozen plasma. By late 1985, 13 treated with cryoprecipitate and three treated with fresh frozen plasma were switched to heat-treated factor VIII or factor IX concentrate, respectively. There were no seroconversions in any of these patients, and for purposes of this study they are classified by their previous blood product exposure, ie, cryoprecipitate or fresh frozen plasma. Those patients classified as cryoprecipitate-treated or fresh frozen plasma-treated received only those products; they were never treated with lyophilized concentrates. Once a patient began lyophilized concentrate, he was classified as a concentrate-treated patient. The patients were classified according to the Public Health Service classification system for HIV infection: acute infection, class I; asymptomatic infection, class II; lymphadenopathy, class III; and AIDS, AIDS-related complex (ARC), neurologic symptomatology, herpes zoster, or oral candidiasis, class IV. Lymphadenopathy is defined as lymph node enlargement (≥1 cm) in greater than two extranodal areas for greater than 3 months.

**Plasma samples.** Citrated plasma samples were obtained from all 181 patients during routine clinical evaluation. In addition, 77 of the 82 HIV seropositive hemophiliacs on whom samples were available annually (66 [86%]) or biannually (11 [14%]) between 1977 and 1986 constituted the population base for determining seroconversion rate and AIDS incidence. The remaining five (6%) HIV antibody–positive hemophiliacs were known to be positive in 1981 (one), 1983 (one), and 1985 (three); however, the last negative sample was either 3 or more years prior to unavailable, and thus these five patients were not included in seroconversion determination. Four were asymptomatic and one had ARC. The group of 79 seropositive hemophiliacs was selected solely on the basis of seroposi-
tivity (HIV antibody positivity) and availability of serial samples; they did not differ from the other HIV seropositive hemophiliacs in age, race, or blood product usage. Further, only one sample per year per patient was used to determine seroconversion. When more than one sample was available, the latest available sample in a given year was the one used for determining seroconversion. Because dates of seroconversion were determined by yearly samples, they may underestimate the date of seroconversion by 6 months or more.

Viral antibody tests. Antibody (IgG) to HIV was measured by an enzyme-linked immunosorbent assay (ELISA) technique using inactivated HTLV–III antigen from the H9/HTLV–III B cell line (Abbott Laboratories, Chicago) or using inactivated whole LAV antigen growth in the CEM–F cell line (Genetic Systems, Seattle). All samples with positive HIV antibody tests were subjected to Western blot confirmation performed at Abbott Laboratories or at Genetic Systems by standard techniques.9

Helper/Suppressor ratios. T lymphocytes, including T helper and T suppressor cells, were determined on a Spectrum III Cytofluorogram, using OKT{sub}T{sub}4, OKT{sub}8, and OKT{sub}T{sub}c antibodies (Ortho Diagnostic Systems, Inc, Raritan, NJ).9

T colony assay. The T colony assay was performed by techniques previously described.16,17 Mononuclear cells were isolated from heparinized samples with Ficoll–hypan and washed in Hank's balanced salt solution (HBSS). They were then incubated in RPMI 1640 media, containing 10% pooled human AB serum, with phytohemagglutinin (PHA) 5 {mu}L per plate, in 35-mm gridded plastic petri dishes, for seven days in a 5% CO{sub}2, 37{degree}C humidified atmosphere (PHA, Wellcome Research Laboratories, Research Triangle Park, NC). Colonies were enumerated with an inverted microscope, with tight clusters of at least 25 cells scored as colonies.

The same technique was repeated with the addition of interleukin-2 (IL-2) (Electronucleonics Laboratories, Silver Spring, MD), at a concentration of 5% per plate. Simultaneously performed controls consisted of 17 healthy heterosexual individuals.

Statistics. The T{sub}4 number of hemophiliacs with and without AIDS was compared by chi-square analysis.

RESULTS

We previously reported, on the basis of ELISA testing, that 80 of 190 hemophiliacs were HIV antibody-positive in 1984.4 Subsequent analysis of these sera by Western blotting indicated that nine of these were false-positive; as such, the adjusted prevalence of antibody positivity was 37.4% (71 of 190). These nine included one treated with factor VIII concentrate, one treated with factor IX concentrate, and seven treated with cryoprecipitate. Of the 181 available for repeat study in 1986 (nine moved), a total of 82 were HIV antibody-positive (all Western blot confirmed), for a prevalence of 45.3%. The HIV seropositive group included 50% (74 of 149) of patients with hemophilia A, of whom 95% were severe (<0.01 U/mL factor VIII) and 5% were moderate (0.01 to 0.05 U/mL), and 25% (eight of 32) of patients with hemophilia B, of whom 88% were severe and 12% moderate. By treatment (Table 1), the HIV seropositive included 82% (63 of 77) of those receiving factor VIII concentrate, of whom 97% were severe and 3% moderate; 48% (13 of 27) of those receiving factor IX concentrate, of whom 92% were severe and 8% moderate; 10% (six of 60) of those receiving cryoprecipitate, of whom 67% were severe and 33% mild; and none of those receiving fresh frozen plasma.

Ten new HIV seroconversions occurred between 1984 and 1986 (in addition to one 1984 seroconversion detected in a previous study).4 These included three treated with factor VIII concentrate, five treated with factor IX concentrate, and two treated with cryoprecipitate. These occurred before switching to heat-treated products in all but one who was the recipient of an unscreened, heat-treated lot of factor VIII concentrate subsequently recalled. In addition, five other patients, on whom serial samples were unavailable, were found to be HIV–seropositive: one in 1981, one in 1983, and three in 1985. Thus, by 1986, there was an 11.7% increase in HIV seroprevalence in those treated with factor VIII concentrate, an 80.1% increase in those treated with factor IX concentrate, and a 96.1% increase in those treated with cryoprecipitate, as compared with 1984 figures. Of the 16 HIV–seronegative hemophiliacs (13 cryoprecipitate-treated; three fresh frozen-plasma treated) who have received solely heat-treated, HIV antibody-screened concentrates since 1985, none have seroconverted (12 or more months follow-up).

Based on seroconversion data on 77 serially sampled HIV antibody-positive hemophiliacs (1977 through 1986), the earliest seroconversion occurred in 1978, and the peak occurred in 1982 (Table 2). Half or more of those treated with factor VIII concentrate who seroconverted did so in 1982 (44 of 60), whereas half or more of those treated with factor IX concentrate (six of 12) or cryoprecipitate (three of five) who seroconverted, did so in 1983.

When the CDC HIV classification system7 was applied to these 79 HIV seropositive hemophiliacs, 35% were class IV, 12% were class III, and 53% were class II (Table 3). Of those seropositive 6 or more years (ie, seroconverting in 1978, 1979, 1980), half have developed AIDS; the cumulative AIDS incidence for those seropositive 5 or more years is 32% (Table 3). The overall AIDS incidence based on all seropositive hemophiliacs in this cohort (n = 82) is 12% (ten of 82) and, based on all hemophiliacs (seropositive and seronegative) in this cohort (n = 181), is 5.5% (ten of 181).

The immunologic changes before, during, and after HIV seroconversion in 63 HIV antibody-positive hemophiliacs, on whom the date of seroconversion was known and who were studied in 1983, 1985, and/or 1986, are shown in Fig 1. Before HIV exposure, the absolute T{sub}4 lymphocyte number

<table>
<thead>
<tr>
<th>Blood Product</th>
<th>1984* (n = 190)</th>
<th>1986 (n = 181)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIII</td>
<td>60/82 (73.2%)</td>
<td>63/77 (81.8%)</td>
</tr>
<tr>
<td>FIX</td>
<td>8/30 (26.7%)</td>
<td>13/27 (48.1%)</td>
</tr>
<tr>
<td>CRYO</td>
<td>3/59 (5.1%)</td>
<td>6/60 (10.0%)</td>
</tr>
<tr>
<td>FFP</td>
<td>0/19 (0%)</td>
<td>0/17 (0%)</td>
</tr>
</tbody>
</table>

Abbreviations: FVIII, factor VIII concentrate; FIX, factor IX concentrate; CRYO, cryoprecipitate; FFP, fresh frozen plasma.

*Revised for one FVIII-treated, one FIX-treated, and seven Cryo-treated who were originally HIV antibody-positive (1984), but on subsequent testing were found to be Western blot negative.
Table 2. HIV Seroconversion Rate and Blood Product Treatment in 77 HIV Antibody-Positive Hemophiliacs

<table>
<thead>
<tr>
<th>Year of Seroconversion</th>
<th>No. Becoming Ab(+)</th>
<th>Blood Product Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. remaining Ab(-)</td>
<td>FVIII</td>
</tr>
<tr>
<td>1977</td>
<td>0/77 (0%)</td>
<td>—</td>
</tr>
<tr>
<td>1978</td>
<td>2/77 (2.6%)</td>
<td>1</td>
</tr>
<tr>
<td>1979</td>
<td>2/75 (2.7%)</td>
<td>2</td>
</tr>
<tr>
<td>1980</td>
<td>2/73 (2.7%)</td>
<td>2</td>
</tr>
<tr>
<td>1981</td>
<td>13/71 (18.3%)</td>
<td>13</td>
</tr>
<tr>
<td>1982</td>
<td>28/58 (48.3%)</td>
<td>26</td>
</tr>
<tr>
<td>1983</td>
<td>19/30 (63.3%)</td>
<td>13</td>
</tr>
<tr>
<td>1984</td>
<td>3/11 (27.3%)</td>
<td>1</td>
</tr>
<tr>
<td>1985</td>
<td>7/8 (87.5%)</td>
<td>1</td>
</tr>
<tr>
<td>1986</td>
<td>1/1 (100%)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>12</td>
</tr>
</tbody>
</table>

(78%) (16%) (6%)

The five HIV antibody-positive hemophiliacs who were not included in this table because their date of seroconversion was not known included three receiving FVIII (HIV Ab(+)) in 1981, 1985, 1985), one receiving FIX (HIV Ab(+)) in 1983), and one receiving cryo (HIV Ab(+)) in 1985) (see text).

A marked decline in T lymphocyte function, measured by T colony growth in the T colony assay, was observed following seroconversion, reaching 1193 ± 537 colony counts by 5 years after seroconversion, and 377 ± 231 colony counts in eight patients at diagnosis of AIDS.

DISCUSSION

This study indicates that the overall prevalence of HIV antibody in hemophiliacs has increased significantly since 1984, with 82% of factor VIII concentrate-treated, 48% of factor IX concentrate-treated, and 10% of cryoprecipitate-treated patients now HIV seropositive. The small number of hemophiliacs who have recently (since mid-1984) seroconverted (11 in this center) occurred before their being switched to screened, heat-treated concentrates or screened single-donor products. Further, none of the 16 previously HIV-seronegative hemophiliacs who have now received solely screened, heat-treated concentrates at this center since 1985 (12 or more months follow-up) have seroconverted. These data suggest that, although new seroconversions have continued to occur since 1984, future exposure to HIV in hemophiliacs is declining and should disappear as a result of HIV antibody screening of donors and heat-treatment of blood products. Thus, hemophiliacs who are HIV antibody-negative would appear to have very minimal risk of future HIV exposure.

The 5.5% AIDS incidence in this hemophiliac cohort (including both HIV-seropositive and HIV-seronegative) is almost four times higher than the national average of 1.5% (303 cases per 20,000 individuals with hemophilia). The reason for this higher incidence in western Pennsylvania is unknown, although not inconsistent with previously described geographic differences in hemophilia AIDS incidence in the United States. The hemophilia AIDS incidence may relate to date of seroconversion, and comparison of peak seroconversion date among hemophilia centers might be extremely helpful in resolving this issue.

The projection for new cases of AIDS among HIV-seropositive hemophiliacs, based on data from this study, is...
that in those seropositive for 5 years or more, the AIDS incidence is 32%. These data coincide with AIDS attack rates for other high-risk groups,6,12 but contrast with the trend reported by a recent CDC survey7 showing a slowing in the rate of hemophilia-associated AIDS over the last five quarters since mid-1985. In interpreting these data, it is important to note the small number of patients in the denominator (19) and the imprecision in exact time of seroconversion. However, because the peak in HIV seroconversion in hemophiliacs in this center and elsewhere occurred in 1982 and 1983,4,5 and because the median latency from infection (seroconversion) to development of AIDS is 5 years or longer, the number of new AIDS cases in hemophiliacs in 1987 or 1988 or later should help resolve this issue.

HIV exposure in hemophiliacs appears to result in a progressive depression of the cellular immune system, with a decline in T lymphocyte function as measured by the T colony assay, and a decline in T4 helper lymphocyte number, with increasing time from seroconversion. Similar defects have been described in other high-risk groups.11,13 Of note in this group, a T4 helper number of less than 100/mm³ was highly predictive of AIDS, with over 60% of those falling below 100/mm³ and none of those remaining above 100/mm³ developing AIDS. It is of interest that before HIV exposure in hemophiliacs, both T4 lymphocyte number and T4/T8 ratio are markedly decreased below control (at a time when T8 is normal). These abnormalities are typical of immunologic alterations previously observed in multitransfused patients.17-26 The causes of these abnormalities are not known, but may be related to chronic exposure to alloantigenic transfused blood proteins or exposure to infectious agents such as cytomegalovirus, hepatitis B, or non-A, non-B hepatitis. Whether these immunologic alterations predispose hemophiliacs to the development of AIDS is unknown.
Following seroconversion, the number of T<sub>S</sub> suppressor lymphocytes remains normal or elevates slightly following seroconversion, consistent with previous reported findings in homosexual men. Thus, an increase in absolute number of T<sub>S</sub> suppressor cells may be another, possibly early, marker of HIV infection.

In summary, the results of this study imply that the AIDS incidence in hemophiliacs is increasing with increasing duration of seropositivity, consistent with previous predictions for other risk groups. Extrapolation from the results of this study, however, should be tempered by the well-described geographic differences in hemophilia-associated AIDS incidence, which may suggest other cofactors in the development of AIDS in this group. These findings further confirm that hemophiliacs were exposed through clotting factor concentrates to infectious viruses, and not just viral antigens. A decline in T lymphocyte function, as measured by the T colony assay, and T<sub>S</sub> number appear to be markers of the immune defect of HIV infection. The pattern of progressive decline in T<sub>S</sub> lymphocytes in HIV-seropositive hemophiliacs over time suggests that, in those in whom the T helper number decreases below 100/mm<sup>3</sup>, there is a high incidence of AIDS.

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