Natural History of Primary Infection With LAV in Multitransfused Patients

By the AIDS–Hemophilia French Study Group

In the course of a prospective study of asymptomatic, multitransfused subjects, seroconversion to human lymphadenopathy-associated virus (LAV/HTLV-III) occurred in 34 hemophilic and in two thalassemic patients. In subjects treated with procoagulant concentrates, primary infection, as evidenced by the development of antibodies to LAV, was a clinically silent event apart from moderate lymph node enlargement in 21% of cases. Concomitant immunologic disturbances mainly affected T lymphocyte subsets. This pattern contrasted with the major lymphadenopathy syndrome observed in the thalassemic patients who received washed erythrocytes from single donors positive for LAV antibodies. Four to 10 months after seroconversion, the incidence of lymphadenopathy reached 46% and the immunologic profile associated inverted T4⁺/T8⁺ lymphocyte ratio and markedly increased serum levels of IgG. In multitransfused hemophilic patients, primary infection with LAV appears to provoke the following simplified sequence of events: decrease of T4⁺ and increase of T8⁺ cell counts preceding or concomitant with the occurrence of IgG LAV antibodies. Polyclonal elevation of IgG and lymph node enlargement occur weeks or months later.

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Seropositive subjects. Forty-five hemophiliacs were seropositive for LAV Ab. Twenty-seven of these with complete records have been included in the prospective study. Nineteen patients with hemophilia A were treated with factor VIII; other patients were treated with factor IX concentrates.

Seronegative subjects. Seronegative patients with hemophilia A received factor VIII over a period of 24 months. During the last 12 months, the products were mostly of French origin. Patients with hemophilia A and antibody to VIII:C received either Autoplex (Travenol Laboratories, Glendale, Calif), FEIBA (Immuno AG, Vienna, Austria), or PPSB (Centre National de Transfusion Sanguine, Paris) over an average of 23.6 months. Patients with hemophilia B were treated over 25.6 months with PPSB concentrate prepared in France. The 16 patients with congenital anemia had received monthly transfusions of packed erythrocytes, amounting to 200 mL/kg/yr.

Table 1. Population Under Study

<table>
<thead>
<tr>
<th>Group</th>
<th>Seronegative</th>
<th>Seroconverter</th>
<th>Seropositive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Treatment (U/kg/m)</td>
<td>No.</td>
</tr>
<tr>
<td>Hemophilia A*</td>
<td>22</td>
<td>53</td>
<td>22</td>
</tr>
<tr>
<td>Hemophilia A with†VIII</td>
<td>12</td>
<td>71</td>
<td>5</td>
</tr>
<tr>
<td>F VIII antibody</td>
<td>10</td>
<td>32</td>
<td>7</td>
</tr>
<tr>
<td>Hemophilia B‡</td>
<td>16</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Chronic anemia§</td>
<td>17.5</td>
<td>(2-62)</td>
<td>16.8</td>
</tr>
<tr>
<td>Mean age (yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Treatment with: *factor VIII concentrates; †activated factor IX preparations; ‡regular factor IX concentrate, P.P.S.B.; §chronic anemia patients received monthly transfusions of packed erythrocytes, amounting to 200 mL/kg/yr.

Seronegative subjects. Seronegative patients with hemophilia A received factor VIII over a period of 24 months. During the last 12 months, the products were mostly of French origin. Patients with hemophilia A and antibody to VIII:C received either Autoplex (Travenol Laboratories, Glendale, Calif), FEIBA (Immuno AG, Vienna, Austria), or PPSB (Centre National de Transfusion Sanguine, Paris) over an average of 23.6 months. Patients with hemophilia B were treated over 25.6 months with PPSB concentrate prepared in France. The 16 patients with congenital anemia had received monthly transfusions of packed erythrocytes, eight of them had undergone splenectomy in the past.

Seroconverted subjects. The total amount of replacement therapy of these patients was calculated by adding the amount of product received during the year prior to the initial checkup and that which was received from the checkup date to the time of first seropositivity. Hemophilia A patients were treated with factor VIII preparations over a period of 21.8 months. Patients with anti-VIII:C antibodies received one or several of the three products mentioned above over a 22-month period. Hemophilia B patients were treated over 21 months. The therapeutic regime of the two thalassemic patients (aged 8 and 16 years) was identical to that of the 16 patients mentioned above; splenectomy had been performed in one.

Methods

Physical examinations were done blind with regard to the patient's anti-LAV status. Lymphadenopathy is defined as two extraglandular areas with at least one lymph node > 1 cm in diameter. Complete blood cell counts were performed on the model S counter (Coulter) and manually on a few occasions. T lymphocyte-membrane associated phenotypes were determined by indirect immunofluorescence using monoclonal antibodies (Ortho, Raritan, NJ) to helper/inducer (OKT4 + ) and suppressor/cytotoxic (OKTR - ) T cell subsets. Absolute number of cells in each subset was calculated as the proportion of positive cells times the peripheral blood lymphocyte count. Normal values for subjects > 10 years of age were 845 ± 110 and 533 ± 129/μL for T4 + and T8 + cells, respectively. In seronegative subjects, these values at two consecutive workups were 1,081 ± 473 and 698 ± 257, respectively. Delayed cutaneous hypersensitivity (DH) to seven antigens was tested with the Merieux Multitest (Lyon, France). For each antigen, the DH was considered positive when the diameter of the induration was at least 2 mm at 48 hours. Because nearly all subjects had been immunized with BCG, diphtheria and tetanus toxoids, DH was considered decreased when positive result was recorded for fewer than two recall antigens.

Virologic serum markers for hepatitis B, Epstein-Barr virus, and cytomegalovirus (HBV, EBV, CMV) were carried out by standard techniques. Coded samples were tested for IgG LAV Ab according to a slightly modified previously described enzyme-linked immunosorbent (ELISA) method. Controls were run with plates coated with uninfected cells. For reactive sera, antibody titers were determined by comparing serial dilutions of test sample to a reference curve. Anti-LAV antibodies of seroconverters were characterized by an immunoblot technique using a standardized viral preparation and, in some instances, by a radioimmunoprecipitation assay. Data were analyzed by the two-tailed t test for unpaired samples and, for comparing proportions, by chi-square statistics or Fisher's exact test.
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RESULTS

Two of 18 seronegative subjects with chronic anemia seroconverted during the course of the prospective study. Details concerning these cases have already been reported, along with the presentation of another thalassemic patient who had his primary LAV infection a few weeks prior to the onset of the present study; this subgroup will not be further analyzed. Among 78 hemophiliacs, who were seronegative at entry and monitored at regular intervals, 34 (44%) seroconverted during the 11 to 17 months of follow-up; this corresponds to a 37% annualized rate of seroconversion. There was no significant difference at the 5% level in the prevalence of seroconversion between hemophiliacs patients treated with factor VIII or factor IX preparations. However, seroconverters treated with factor VIII or factor IX concentrates had received more products than their respective serologically negative controls ($P < .05$) (Table 1).

Irrespective of their type of therapy, seronegative hemophiliac patients had few abnormalities of index parameters. Two initially had an association of immunologic abnormalities: low T4* and elevated T8* counts, T4*/T8* ratio <0.50 and serum IgG level above 1,600 mg/dL. This pattern was suggestive of primary infection with LAV, and additional tests were performed to seek evidence for viral transmission; the tests failed to detect LAV Ab. The other 42 subjects (Table 2) had dissociated abnormalities. Eleven patients had T4* counts <600/μL, causing a T4*/T8* ratio <1.00 in six patients.

The occurrence of abnormalities of the eight index parameters in seroconverters was studied. For all patients, a checkup at 5.3 ± 1.8 months prior to seroconversion (in 11 subjects, it corresponded to the initial checkup) and at seroconversion were available; for 24 patients data, obtained at 5.3 ± 1.5 months postseroconversion were also analyzed (Table 2). Both the actual number of results outside normal ranges and differences from initial checkup indicate the presence of clinical and/or biological abnormalities in 19 patients several months prior to detection of anti-LAV IgG.

These were essentially decreased T4* cell count, T4*/T8* ratio, and reactivity to skin DH tests associated with elevation of T8* cell count and IgG level. When these subjects were compared with the group of patients who remained seronegative, it was difficult to evaluate whether the abnormalities were related to patients infected with LAV but still seronegative or to unspecific immunologic disturbances.

At seroconversion, lymphadenopathy was present in 21% of the patients, and the abnormalities seen before seroconversion were either similar or increased in number or magnitude. When differences from initial status were considered, ten subjects had significant changes in T lymphocyte subset counts related to either decreased T4* count or increased T8* count and (only once) to concomitant decrease of T4* and increase of T8* cells. The mean level of serum IgG was significantly higher than at entry (1,490 ± 460 μg/dL, $P < .001$ to level at seroconversion). Only two seroconverters remained normal for all index parameters throughout the observation period. Serum IgM levels were similar before and at seroconversion (195 ± 86 mg/dL and 194 ± 114, respectively). A slight decrease remaining below the level of significance was observed after seroconversion (166 ± 50 mg/dL).

Data obtained several months after seroconversion are remarkably similar to those observed in the 27 patients who were seropositive at the onset of the study and remained so over the 14 months of follow-up (Table 2). In this group, the incidence of lymphadenopathy, inverted T4*/T8* ratio, and elevated IgG levels were 52%, 48%, and 59%, respectively.

The sequential occurrence of enlarged lymph nodes and elevated serum IgG levels in the seroconverters is shown in Fig 1. Overall, it appears that in a previously healthy patient with hemophilia, the occurrence of lymphadenopathy with concomitant decreased T4* cell count or increased T8* cell count, inverted T4*/T8* ratio, and elevated serum IgG level are strongly suggestive of LAV infection. In only one patient, a flu-like syndrome was noticed in the weeks preceding

![Fig 1. Checkups for lymph nodes (○) and IgG levels (Δ) in seroconverters. The presence of enlarged lymph nodes in at least two extraglandular areas or IgG level above 1,600 mg/dL is indicated by closed symbols. SC denotes seroconversion. Intervals before and after SC are defined as in Table 2.](image-url)
seroconversion. All other patients seroconverted asymptotically aside from small-size lymphadenopathy.

IgG antibodies to LAV were analyzed by ELISA, immunoblot and, in some cases, radioimmunoprecipitation assay (RIPA) (Table 3). Variations in ELISA titers were observed from patient to patient. For a given subject, titers at seroconversion and in postseroconversion samples were stable, and the median titer was 1:800 in both series. However, in two patients, the titer increased significantly between the two samplings (1:100 to 1:1,600 and 1:200 to 1:1,600, respectively), which may indicate that the first blood specimen was drawn during an ongoing rise of IgG antibodies. ELISA-positive samples were checked by immunoblotting. All but one sample reacted with one or several LAV constitutive proteins. Analysis by RIPA, undertaken for selected samples, further revealed antibody to gp 110 in all 11 samples, including the ELISA-positive, Western blot-negative, second sample of patient 3. In ten postseroconversion sera, additional antibody specificities were detected, mainly against p34. No obvious pattern emerged from comparison of clinical and biological status of patients with antibody titers and/or specificities.

DISCUSSION

The population included in this prospective study was selected on the basis of normality of clinical, hematologic, and immunologic criteria. As expected, although it provided a standard background, the incidence of LAV Ab-positive subjects was lower (35%) than the general incidence observed in the cross-section study (47%), due to the LAV Ab testing which took place several months after the prospective study was launched. The advantage was a relative homogeneity of the seronegative subjects, of whom 44% seroconverted.

Primary LAV infection caused by treatment of hemophilic patients with factor VIII or IX preparations was a clinically silent event. The only clinical symptom was a moderate enlargement of peripheral lymph nodes. Data obtained from seronegative subjects indicated that, in this population in which 50% of the subjects were <18 years of age, some isolated lymphadenopathy may have other causes. This feature differs strikingly from reports concerning LAV seroconversion related to LAV Ab-positive washed RBCs, whole blood, and plasma, in which massive multifocal lymphadenopathy similar to LAS is described. Among the 18 patients with congenital anemias enrolled in this study, two 8-year-old boys seroconverted. Both presented with large-size lymphadenopathy in all areas, similar to a patient already reported who seroconverted before entry. In addition, although no other symptom was recorded, one of the two boys had a significant weight loss (4.5 kg) between the time of entry and seroconversion 9 months later. The acute mononucleosis-like syndrome described after accidental needle-stick transmission and in a prospective study among Australian homosexuals seems to correspond to the complete clinical picture of LAV primary infection. In our study, the progressive increase of the prevalence of lymphadenopathy before, during, and postseroconversion and their

Table 3. Follow-up of IgG LAV Ab in Seroconverters

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Screening</th>
<th>ELISA</th>
<th>Immunoblot</th>
<th>RIPA</th>
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<tr>
<td></td>
<td></td>
<td>Titer</td>
<td>p18</td>
<td>p25</td>
</tr>
<tr>
<td>3</td>
<td>SC</td>
<td>+</td>
<td></td>
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<tr>
<td></td>
<td>4 mos</td>
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<tr>
<td>4</td>
<td>SC</td>
<td>+</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 mos</td>
<td>+</td>
<td>200</td>
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</tr>
<tr>
<td>11</td>
<td>SC</td>
<td>+</td>
<td>1,600</td>
<td>+</td>
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<td></td>
<td>4 mos</td>
<td>+</td>
<td>1,600</td>
<td>+</td>
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<td>12</td>
<td>SC</td>
<td>+</td>
<td>1,600</td>
<td>+</td>
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<tr>
<td></td>
<td>8 mos</td>
<td>+</td>
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<td>+</td>
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<td>SC</td>
<td>+</td>
<td>200</td>
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<td>8 mos</td>
<td>+</td>
<td>1,600</td>
<td>+</td>
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</tbody>
</table>

ELISA, enzyme-linked immunosorbent assay; SC, seroconversion sample. Months are the number of months post-SC for the follow-up sample.

*Patients 3 through 14 were treated with factor VIII preparations; patients 18 through 24 received factor IX concentrates.

†Viral proteins p18, p25, p34, and p41 were identified as described in the Materials and Methods section.

‡RIPA denotes radio immunoprecipitation assay performed on 11 ELISA-positive samples; gp 110: membrane glycoprotein, (+) expresses a weak antigen-antibody reaction.
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Persistence over at least 5 months is strongly suggestive of inducement by LAV infection. Similar findings have previously been reported in hemophilic populations.15-18

Immunologic disturbances induced by replacement therapy with factor VIII concentrates in seronegative cohorts of hemophiliac patients have been described.15-19 In this study, elevation of T suppressor cells and sharp rise in IgG serum levels appeared related to the LAV infection (Table 2). This observation made in the seroconverters was confirmed by our findings in the seropositive group of patients. Persistent lymphadenopathy and elevation of T8+ cells and serum IgG levels may thus be considered as reliable markers of LAV infection, particularly when they are associated (Fig 1). However, any of these three parameters can be separately altered over time in a given subject. In contrast, low T4+ cell counts were randomly distributed among seronegative and seropositive groups. A moderate decrease in T4+ may therefore reflect in part the effects of replacement therapy itself. T helper cells are known to be privileged target of LAV; our data, however, did not single out a specific defect of this subset, which seems more prone to abnormalities related to other causes, such as EBV, HBV, or CMV chronic infections. Repeated evaluation of delayed hypersensitivity for seven antigens by the Multitest appeared to be a useful tool for evaluation of immune reactivity. Although this repetition has been reported to modify the extent of response, the number of responsive antigens was not affected.9

From the collected data, a simplified sequence of event appears to be: decrease in T4+ and increase of T8 counts preceding or concomitant with occurrence of IgG LAV Ab, followed by polyclonal elevation of circulating IgG and lymph node enlargement (Fig 2).

Our data confirm previous findings demonstrating that seroconversion is not a reflection of an immunization by incomplete noninfective viral antigens but rather is a replicating virus hosted by T lymphocytes.20 It is possible that the mild primary infection syndrome observed in hemophilic patients is caused by a small number of infective particles deriving from few donors whose plasma is highly diluted in a large pool. A possible attenuation of viral pathogenicity related to purification procedures and freeze-drying may also play a role. Whether such a small infective dose carries a better long-term prognosis remains to be evaluated.

ACKNOWLEDGMENT

We wish to thank A. Catillon for expert secretarial help and J. P. Boina for his assistance in computerized analysis.

REFERENCES


Fig 2. Schematic diagram picturing the sequential values of T lymphocyte subset counts, serum IgG levels (patients < 10 years of age omitted), and prevalence of lymphadenopathy (LAS) in seroconverting hemophiliac patients. Bar (top) corresponds to the likely period of LAV contamination. Means of data from 24 patients > 10 years of age were used to draw this diagram.


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