CONCISE REPORT

LAV/HTLV III Presence in Peripheral Blood Lymphocytes of Seropositive Young Hemophiliacs

By Edward D. Gomperts, Paul Feorino, Bruce L. Evatt, Donna Warfield, Robert Miller, and J. Stephen McDougal

Recent studies indicate a high prevalence of seropositivity to the lymphadenopathy-associated virus/human T-lymphotropic virus (type III) among individuals with hemophilia exposed to clotting factor concentrates prepared from large donor pools. The peripheral blood lymphocytes of 19 young seropositive patients with inherited bleeding disorders were examined for the presence of this virus by coculture with phytohemagglutinin-stimulated lymphocytes. Viral isolates were obtained from six of 19 patients. While none of these patients have developed the acquired immunodeficiency syndrome (AIDS) or AIDS-related complex, five of them had lymphadenopathy in two noncontiguous areas, and two showed clinically symptomatic enlarged tonsils and adenoids. Of the 13 patients in whom virus was not demonstrated, five were judged clinically normal and five had mild lymphadenopathy in one anatomical area. These results suggest that as many as 33% of hemophiliacs (six of 19 patients studied), who have circulating antibodies to mature viral proteins, have viral-infected peripheral blood lymphocytes capable of infecting other lymphocytes in vitro.

THE ACQUIRED immunodeficiency syndrome (AIDS) was first reported in individuals with hemophilia in July 1982. Shortly after this observation, altered immune status in patients with hemophilia was documented, and increasing numbers of hemophilic patients with AIDS have been observed, in both the United States and Europe. More recently, a retrovirus has been isolated both in France (lymphadenopathy-associated virus [LAV]), and in the United States (human T-lymphotropic virus type III [HTLV III]). A high prevalence rate of antibodies to LAV/HTLV III has been demonstrated in various high-risk groups, including hemophiliacs. It is now believed that LAV/HTLV III virus is etiologically associated with AIDS.

The question arises as to whether individuals who have antibodies to LAV/HTLV III, and have therefore been infected with the virus, continue to have virus present in circulating lymphocytes concurrent with the presence of antibody. We have cocultured phytohemagglutinin (PHA)-stimulated peripheral blood lymphocytes from a group of young patients with hemophilia with known seroconversion since 1978 to determine the presence of the LAV/HTLV III virus.

MATERIALS AND METHODS

Patient Population

Nineteen young patients (mean age, 15 years; range, 8 to 26 years) with clinically severe hemophilia A (16 patients), hemophilia B (two patients), and von Willebrand's disease (one patient) were studied. All patients had received treatment with commercial clotting factor concentrates, although one child with von Willebrand's disease had also received cryoprecipitate to control bleeding episodes. All patients had been enrolled in a Hemophilia Comprehensive Care Center and had been followed longitudinally at this Center since 1978. Eighteen patients had demonstrated antibody seroconversion to the LAV by Western blot assay in stored serum or plasma samples dating from 1978 through 1984. The mean known duration of a-LAV/HTLV III seropositivity for the group was 18 months, with a range of five to 74 months. One child showed a-LAV/HTLV III positivity in the sample collected in 1978 when he was 26 months of age and in all subsequent samples evaluated.

The research procedure and informed consent for the study was approved by the institutional human research review committee. After informed consent was obtained, the patient was physically examined and blood was drawn for a full blood count, platelet count, and T cell subset analysis, and an aliquot was obtained for lymphocyte culture studies. Lymphadenopathy was considered clinically significant when palpable nodes were detected in two or more noncontiguous areas, specifically cervical/axillary or bilateral axillary nodal groups. A palpable spleen was not considered an LAV/HTLV III-relevant clinical change. Tonsils and adenoids were not labeled "enlarged" unless they were clinically seen to be enlarged and there was associated symptomatology including symptoms on swallowing and/or nasal speech in consequence.

Methods

Western blot assay. Western blots were performed by the method of Tsang et al. Viral antigens for Western blots were prepared by ultracentrifugation of LAV/HTLV III-infected culture supernates over a 30% wt/wt sucrose cushion (80,000 g for one hour). The pellets were dissolved in 0.01 mol/L Tris, pH 8.0, containing 1% sodium dodecyl sulfate, 25 μg/mL bromphenol blue, 10% glycerol, and 5% 2-mercaptopethanol, and heated at 65 °C for 30 minutes.

T cell phenotypes were determined by indirect immunofluorescence with a fluorescence-activated cell sorter using commercial monoclonal antibodies (OKT3, OKT4, and OKT8, Ortho Pharmaceutical Corp, Raritan, NJ) as described by Nicholson et al. Viral isolation and serology. The virus isolation techniques have been described previously. Briefly, thawed lymphocytes were cocultivated with 3-day-old PHA-stimulated normal human lymphocytes from a group of young patients with hemophilia with known seroconversion since 1978 to determine the presence of the LAV/HTLV III virus.
phocytes in RPMI 1640 medium containing 5% interleukin 2. Additional normal human lymphocytes were added every four to five days. The cultures were monitored for virus replication by immunofluorescence and particulate reverse transcriptase (RT) assays at intervals for 28 days. Concentrated culture fluids were assayed for particulate RT activity with synthetic template primer (A),(dt)2,8 and either 7.5 nmol/L Mg2+ or 0.1 nmol/L Mn2+ as cation.13 The direct immunofluorescence test was performed with a high-titer a-LAV/HTLV III human serum.13 All cultures were examined by thin-section electron microscopy (EM). Cells were fixed for EM with 2.5% glutaraldehyde, post-fixed with osmium tetroxide, embedded in epon-araldite, and stained with lead citrate and uranyl acetate. Cultured cells were considered infected if they produced RT in the supernatant fluid, expressed LAV/HTLV III-specific antigens, and contained LAV/HTLV III-like virus particles when examined by EM.15 Primary cultures of four different batches of normal human lymphocytes used for cocultivation were monitored similarly to ensure absence of primary infection or laboratory contamination.

RESULTS

LAV/HTLV III virus was detected in PHA-stimulated and propagated lymphocytes in six of the 19 patients (Table 1). The RT maximum activity was detected between days 12 and 18 of culture (mean, 15 days), with counts reading between 115,000 and 583,000 (mean, 252,000) as compared with normal tissue culture controls of between 1,500 and 2,100 (mean, 1,900). Those samples classified as negative showed maximum activity under 5,000 counts. None of these patients showed the AIDS or AIDS-related complex (ARC) at the time of investigation, although five of the six patients had lymphadenopathy, specifically palpable cervical and axillary, or bilateral axillary lymph node enlargement. In addition, two of these patients showed enlarged tonsils and adenoids. None of these patients had any abnormality in platelet or absolute lymphocyte numbers, and five of six patients demonstrated an altered T helper/suppressor cell (Th/9) ratio. One patient positive for circulating virus showed no clinical changes.

Of the 13 patients in whom the virus was not demonstrated, five patients were judged clinically normal; five patients were considered to have lymphocytic lymphoma; and three of these patients demonstrated altered T helper/suppressor cell (Th/9) ratio. One patient positive for circulating virus showed no clinical changes.

Table 1. Details of Serologic, Clinical Laboratory, Virologic, and Clinical Observations Made on 19 Patients Who Show Antibodies to the LAV/HTLV III Virus

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Year of Birth (Factor Deficiency)</th>
<th>LAV/HTLV III Culture (Max RT Counts/dl)*</th>
<th>Date a-LAV/HTLV III First Detected</th>
<th>Absolute Lymphocyte Count (uL)</th>
<th>Platelet Count (uL)</th>
<th>OKT4/8</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1973 (VIII)</td>
<td>+ (115,000/18)</td>
<td>11/82</td>
<td>2,000</td>
<td>179,000</td>
<td>0.83</td>
<td>Mild cervical and axillary lymphadenopathy; spleen tip; HBsAg*</td>
</tr>
<tr>
<td>2</td>
<td>1970 (VIII)</td>
<td>+ (124,000/14)</td>
<td>6/83</td>
<td>1,517</td>
<td>275,000</td>
<td>0.77</td>
<td>Mild axillary and cervical lymphadenopathy</td>
</tr>
<tr>
<td>3</td>
<td>1959 (VIII)</td>
<td>+ (218,000/16)</td>
<td>3/83</td>
<td>1,511</td>
<td>200,000</td>
<td>0.44</td>
<td>No abnormality</td>
</tr>
<tr>
<td>4</td>
<td>1971 (VIII)</td>
<td>+ (330,000/14)</td>
<td>12/82</td>
<td>1,938</td>
<td>282,000</td>
<td>0.39</td>
<td>Inhibitor; mild cervical and axillary lymphadenopathy; spleen 1/2 cm; tonsil enlargement</td>
</tr>
<tr>
<td>5</td>
<td>1972 (VIII)</td>
<td>+ (144,000/16)</td>
<td>2/84</td>
<td>1,932</td>
<td>275,000</td>
<td>1.19</td>
<td>Mild axillary lymphadenopathy (bilateral); spleen tip</td>
</tr>
<tr>
<td>6</td>
<td>1974 (VWD)</td>
<td>+ (583,000/12)</td>
<td>5/83</td>
<td>1,976</td>
<td>264,000</td>
<td>0.88</td>
<td>Spleen tip; mild cervical and axillary lymphadenopathy; tonsils and adenoids enlarged</td>
</tr>
<tr>
<td>7</td>
<td>1976 (VIII)</td>
<td>– (&lt;5,000)</td>
<td>9/78</td>
<td>3,640</td>
<td>273,000</td>
<td>0.65</td>
<td>Low responder inhibitor; mild cervical and axillary lymphadenopathy; spleen tip; enlarged tonsils</td>
</tr>
<tr>
<td>8</td>
<td>1976 (VIII)</td>
<td>– (&lt;5,000)</td>
<td>6/84</td>
<td>1,920</td>
<td>262,000</td>
<td>0.77</td>
<td>No abnormality</td>
</tr>
<tr>
<td>9</td>
<td>1964 (VIII)</td>
<td>– (&lt;5,000)</td>
<td>11/83</td>
<td>1,156</td>
<td>192,000</td>
<td>0.78</td>
<td>Mild hypertension; spleen tip</td>
</tr>
<tr>
<td>10</td>
<td>1971 (VIII)</td>
<td>– (&lt;5,000)</td>
<td>7/83</td>
<td>1,848</td>
<td>318,000</td>
<td>0.59</td>
<td>Spleen tip</td>
</tr>
<tr>
<td>11</td>
<td>1968 (VIII)</td>
<td>– (&lt;5,000)</td>
<td>5/83</td>
<td>1,961</td>
<td>358,000</td>
<td>0.79</td>
<td>Mild axillary lymphadenopathy (unilateral); spleen tip</td>
</tr>
<tr>
<td>12</td>
<td>1976 (VIII)</td>
<td>– (&lt;5,000)</td>
<td>7/83</td>
<td>2,556</td>
<td>337,000</td>
<td>0.87</td>
<td>No abnormality</td>
</tr>
<tr>
<td>13</td>
<td>1963 (VIII)</td>
<td>– (&lt;5,000)</td>
<td>9/83</td>
<td>3,074</td>
<td>150,000</td>
<td>0.73</td>
<td>No abnormality</td>
</tr>
<tr>
<td>14</td>
<td>1974 (VIII)</td>
<td>– (&lt;5,000)</td>
<td>2/84</td>
<td>2,808</td>
<td>291,000</td>
<td>0.50</td>
<td>No abnormality</td>
</tr>
<tr>
<td>15</td>
<td>1966 (VIII)</td>
<td>– (&lt;5,000)</td>
<td>8/83</td>
<td>3,233</td>
<td>210,000</td>
<td>0.63</td>
<td>No abnormality</td>
</tr>
<tr>
<td>16</td>
<td>1958 (VIII)</td>
<td>– (&lt;5,000)</td>
<td>1/84</td>
<td>512</td>
<td>74,000</td>
<td>0.61</td>
<td>HBsAg*; hypersplenism</td>
</tr>
<tr>
<td>17</td>
<td>1974 (VIII)</td>
<td>– (&lt;5,000)</td>
<td>4/83</td>
<td>2,048</td>
<td>234,000</td>
<td>0.86</td>
<td>Spleen tip</td>
</tr>
<tr>
<td>18</td>
<td>1959 (IX)</td>
<td>– (&lt;5,000)</td>
<td>5/83</td>
<td>1,404</td>
<td>146,000</td>
<td>0.50</td>
<td>Chronic renal failure; mild axillary lymphadenopathy</td>
</tr>
<tr>
<td>19</td>
<td>1967 (IX)</td>
<td>– (&lt;5,000)</td>
<td>4/84</td>
<td>2,016</td>
<td>290,000</td>
<td>0.63</td>
<td>Mild cervical and axillary lymphadenopathy</td>
</tr>
</tbody>
</table>

*a-LAV/HTLV III, antibodies to the LAV/HTLV III virus; vWD, von Willebrand’s disease.  
*Maximum reverse transcription counts and the day on which maximum activity was observed.  
†Spleen edge palpable at the left costal margin.
mal, five patients showed a palpable spleen with or without one group of palpable nodes, and two patients were considered to have lymphadenopathy in two noncontiguous areas, one of them showing enlarged tonsils and adenoids. Two patients in this group had platelet counts below normal.18 One of these patients also showed an absolute lymphopenia. His clinical presentation is complicated by HBsAg-associated cirrhotic changes and hypersplenism; the second patient with mild thrombocytopenia has chronic renal failure and has been managed with hemodialysis since 1980. All of these 13 patients showed T_h/α ratios below 1.0.

DISCUSSION

This study indicates that a substantial number (33% in this series) of hemophiliacs who have circulating antibodies to the LAV/HTLV III also have circulating viral-infected lymphocytes which are capable of infecting other cells in coculture. Although it is unclear to what extent this infective potential may be expressed in person-to-person transmission, this potential is present for as long as nine to 24 months after seroconversion of the patient. None of these patients have demonstrated the clinical manifestations of AIDS or ARC, but they do have mild detectable immune proliferative changes as indicated by persistence of palpable lymph nodes in two or more noncontiguous areas. It is reasonable to expect that this cohort will continue to express clinical changes, serologic positivity, and viral persistence; however, it is not possible to predict whether the AIDS or ARC will become manifest.

The remainder of the patients who were a-LAV/HTLV III positive failed to demonstrate virus on culture. These patients could be subdivided into two groups of approximately equal numbers. One group showed no clinical changes and the second had clinical evidence of a lymphoproliferative response. One of these patients had demonstrable a-LAV in samples stored as early as 1978. At the time of this study, this child showed lymphadenopathy in the anterior and posterior cervical and axillary areas and showed enlarged tonsils and adenoids to the extent that speech was nasal. Clearly, this child demonstrated lymphoproliferative features that suggest continued LAV/HTLV III infection.

Viral studies previously reported in patients with AIDS indicate that infection with virus and serologic positivity may occur concurrently,14,17 and our observations extend this to include seropositive individuals with hemophilia who have mild clinical changes. This study also indicates that there is a wide spectrum of immune and clinical responses to infection with this virus. The ultimate outcome in any of these subgroups is unknown, but on the basis of early observations made on other high-risk groups followed from two to five years after seroconversion, it is reasonable to expect that some will develop AIDS, some will show ARC, some will become asymptomatic carriers, and some will possibly mount a successful immune response with consequent clearance of the viral infection. Only longitudinal studies will clarify the possible long-term outcomes.

Severe hemophiliacs require the frequent use of clotting factor concentrates usually prepared from a pool of plasma collected from as many as 20,000 donors. These patients thus run the risk of reinfection with LAV/HTLV III. It is uncertain what role reinfection with this retrovirus may have on the clinical course of the illness or the ability to detect antibody or isolate the virus in culture. Recent studies have shown that the virus is heat-sensitive and heat treatment may greatly reduce the risk of infection from concentrates.18 Thus, the use of heat-treated factor concentrates appears to be a reasonable approach to therapy of bleeding episodes in hemophiliacs to reduce the potential risk of reinfection.

One important implication of these studies is that a substantial number of asymptomatic seropositive individuals are potentially infectious by the proven routes of blood and sexual transmission.17 Consequently, it is recommended that precautions to prevent person-to-person spread should be taken and include the recommendations that apply to other high-risk groups. For example, these patients should receive regular clinical evaluations, employ the use of condoms for sexual intercourse, and should not share implements such as toothbrushes and razors, which can be contaminated with blood. In addition to these general protective measures, infusion supplies that have been used during home therapy should be safely stored in solid-walled containers until the contents can be autoclaved or incinerated. Any blood spilled during infusions in home therapy should be cleaned with diluted household bleach.

REFERENCES

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17. Center for Disease Control: Provisional Public Health Service inter-agency recommendations for screening donated blood and plasma for antibody to the virus causing acquired immunodeficiency syndrome. MMWR 34:1, 1985
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