CONCISE REPORT

Antibodies to Human T-Lymphotropic Virus Type III (HTLV-III) in Saliva of Acquired Immunodeficiency Syndrome (AIDS) Patients and in Persons at Risk for AIDS

By D.W. Archibald, L. Zon, J.E. Groopman, M.F. McLane, and M. Essex

Whole saliva samples collected from available people at risk in Boston for infection with human T-lymphotropic virus type III (HTLV-III/LAV), from late 1984 through early 1985, were analyzed for the presence of antibodies to viral proteins. Fourteen of 20 (70%) acquired immunodeficiency syndrome (AIDS) patients and 14 of 15 (93%) AIDS-related complex (ARC) patients had salivary antibodies that reacted with the virus-encoded glycoproteins gp160 and gp120 of HTLV-III infected cells. All of the AIDS and ARC patients had serum antibodies to the same antigens. Of 20 sex partners of AIDS/ARC patients, nine (45%) showed anti-HTLV-III antibodies, and four of 18 (22%) healthy homosexual males also were positive for such antibodies.

In the United States and Europe, the acquired immunodeficiency syndrome (AIDS) occurs primarily in sexually active homosexual men, intravenous drug users, and blood product recipients. Heterosexual partners of all of the above have developed AIDS. The apparent routes of transmission of the human T-lymphotropic viruses (HTLV) include close sexual contact, transfusion of blood products, and parenteral exposure. One member of the HTLV family of retroviruses, HTLV-III/LAV, is generally accepted to be the etiologic agent of AIDS. This virus has been isolated from a high percentage of patients with AIDS or AIDS-related complex (ARC), and antibodies to it have been found in 88% to 100% of AIDS patients and high-risk individuals.

Another horizontally transmissible retrovirus, feline leukemia virus (FeLV) is responsible for a series of lymphoproliferative and cytopathic diseases similar to those of the HTLV family. FeLV has been shown to be transmissible via saliva, and it replicates well in the salivary gland acinar cells. Moreover, saliva contains the highest level of infectious particles of any body fluid and is easily passed by mutual grooming in comminually housed cats. Oronasal lymphoid cells are the first cells to show evidence of FeLV infection following an oral inoculation; thus, the upper respiratory tract can also be the portal of viral entry as well as the site of transmission.

HTLV-III infectious particles have recently been isolated from the saliva of four of ten ARC patients and four of six healthy homosexuals, and retroviral particles were observed by electron microscopy in the saliva in one of four AIDS patients tested. These patients were all seropositive for antibodies to HTLV-III. Saliva passed via kissing has also been implicated as a vehicle of transmission in at least one case of HTLV-III infection.

In respiratory viral diseases, the presence of immunoglobulin A (IgA) antibodies, which are found in higher concentrations in mucosal secretions, ie, saliva, correlates better with viral immunity than does the level of antiviral serum antibodies. We report here that antibodies directed to HTLV-III proteins were found in the whole saliva of patients with AIDS and ARC.

MATERIALS AND METHODS

Whole saliva was collected from patients and healthy participants in Boston, during late 1984 and early 1985, and stored at -70 °C before being analyzed under code. Following 1:2 dilution with phosphate buffered saline or water, samples were centrifuged and passed through 0.22 μm pore size filters (Millipore Corp. Bedford, Mass.). Radioimmunoprecipitation of 35S-cysteine-labeled HTLV-III-infected H9/HTLV-III-infected and H9-uninfected cell lysates was performed as previously described except that Protein-A beads were first reacted with 10 μL of sheep anti-human secretory IgA serum (Cappel Laboratories, Inc, Cochranville, Pa) before addition of 150 μL of the saliva samples. Serum from an AIDS patient known to be positive for HTLV-III antigens p24, p55, gp120, and gp160 was used as a positive control. Immunoprecipitates were analyzed in a 10% acrylamide resolving gel with a 3.5% stacking gel, according to the discontinuous buffer systems of Laemmli. Autoradiography was carried out for five days at -70 °C. Serum samples from the same patients were obtained at approximately the same time and were tested by immunoprecipitation as described earlier.

RESULTS

The positive saliva samples reacted with proteins of molecular weights 160,000 and 120,000 that were present in the

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Supported by National Institutes of Health Grants No. CA-18216 and CA37466. D.W.A. is supported by Institutional Research Service Award No. CA-09031.


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infected cell lysate and not in the uninfected cell lysates. These correspond to \textit{env} gene glycoproteins from HTLV-III as previously reported.\textsuperscript{9,17} Salivary antibodies usually did not recognize \textit{gag} viral proteins p55 and p24 that were precipitated by serum from an AIDS patient with known high anti-HTLV-III titers as shown in Fig 1. However, proteins of molecular weight 55,000, 38,000, 27,000, 24,000, and 17,000 were occasionally precipitated by some saliva samples that also contained antibodies to gp160 and gp120. Of 20 AIDS patients, 14 had salivary antibodies, while 14 of 15 ARC patients had salivary antibodies to gp160 and gp120. Nine of 20 healthy sex partners of AIDS and ARC patients were also salivary antibody positive. Two of these partners of AIDS patients had virus isolated from their plasma, but had no serum antibodies and also had no evidence of antibodies in their saliva.\textsuperscript{13} Four of 18 healthy homosexual males were positive for salivary antibodies. As additional controls, ten patients hospitalized for cancer therapy, including five leukemia/lymphoma patients and five carcinoma patients, were also negative. Eleven laboratory workers with exposure to HTLV-III products were similarly negative for salivary antibodies to HTLV-III. A serum antibody positive transfusant patient also had salivary antibodies to HTLV-III proteins, indicating that the gut-associated lymphoid system can be stimulated by systemic HTLV-III infection or that viral replication could occur there.

The presence of salivary antibody to HTLV-III correlated with the serum antibody status except that not all serum antibody positive AIDS patients had salivary antibody. The class of antibody in the saliva that reacted with the virus antigens was IgA, since failure to use an anti-IgA antibody to bind the salivary immunoglobulin led to a greatly diminished reactivity. The data is summarized in Table 1.

**DISCUSSION**

Mucosal IgA is known to be protective against some viral diseases, presumably by blocking viral attachment to and/or entry into cells. FeLV is an exogenous transmissible retrovirus, as is HTLV-III, that can be passed orally via saliva even though the virus cannot survive the pH of the stomach. Therefore, the FeLV retrovirus must be able to enter the mucosal cells of the oropharyngeal region. Mucosal antibodies found in secretions such as saliva would be expected to be at least partially protective against this process. Since infectious HTLV-III has been found in saliva, the possibility exists that mucosal IgA antibodies may play a protective role against virus transmission either by preventing oropharyngeal viral entry or by blocking virus and preventing viral spread via saliva. The envelope directed IgA antibodies found in saliva would be excellent candidates for the blocking action. The oral polio vaccine protects not only by initiating systemic antibodies, but also by stimulation of IgA-producing lymphocytes in the alimentary tract. Reinfection of the gut is greatly reduced by the local IgA immunity.\textsuperscript{18} Similar-
SALIVARY ANTIBOIDS TO HTLV-III

Table 1. Presence of Serum and Salivary Antibodies to HTLV-III

<table>
<thead>
<tr>
<th>Health Status</th>
<th>No. Tested</th>
<th>Serum Antibodies</th>
<th>Salivary Antibodies to gp160/gp120</th>
<th>Salivary Antibodies to p55/p24</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS</td>
<td>20</td>
<td>20</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>ARC</td>
<td>15</td>
<td>14</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>Healthy sex partners</td>
<td>20</td>
<td>9</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>of AIDS/ARC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy homosexuals</td>
<td>18</td>
<td>4*</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Cancer patients</td>
<td>10</td>
<td>NT*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Laboratory workers</td>
<td>11</td>
<td>0*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Healthy transfusion recipient</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviation: NT, not tested.
*Sera from two laboratory workers, one healthy homosexual, and all cancer patients were unavailable for testing.

Table 2. Percentage of Serum and Salivary Antibodies to gp120

<table>
<thead>
<tr>
<th>Health Status</th>
<th>Serum Antibodies</th>
<th>Salivary Antibodies to gp120</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>ARC</td>
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<td>14</td>
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<tr>
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<td>18</td>
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</tr>
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<td>NT*</td>
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<tr>
<td>Laboratory workers</td>
<td>11</td>
<td>0*</td>
</tr>
<tr>
<td>Healthy transfusion recipient</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Thus, salivary antibody levels may drop as salivary viral levels drop. Assuming a normal concentration of 20 mg/100 mL of IgA in whole saliva as compared with 328 mg/100 mL of IgA and 1,230 mg/100 mL of IgG in serum, then there is only 1/80th as much potential antibody in saliva as compared with total serum levels. Salivary IgG levels are usually negligible even when transudation of serum from around the teeth is included.

Gp160 and gp120 are the likely targets of virus neutralizing antibodies. The finding of IgA antibodies directed toward HTLV-III env proteins in saliva also provides an opportunity for studies of IgA in other body fluids, such as semen, vaginal fluids, and tears. The role of these antibodies, in the various secretory fluids, may play in viral transmission has not been evaluated. If salivary IgA antibodies are found to be neutralizing, then questions concerning the strain specificity of antibodies directed toward HTLV-III proteins should be addressed for all mucosal fluids.

Salivary IgA antibodies were detectable in all the subjects (27 of 27) that were positive for serum antibodies except the AIDS patients. This raises the possibility that a screening test for the detection of virus-infected healthy carriers might be conducted using saliva rather than blood.

ACKNOWLEDGMENT

We thank J. Kinniburgh for help in obtaining samples.

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