Salivary Antibodies as a Means of Detecting Human T Cell Lymphotropic Virus Type III/Lymphadenopathy-Associated Virus Infection

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Of 45 individuals seropositive for human T cell lymphotropic virus type III/lymphadenopathy-associated virus, 45 were found to have detectable salivary antibodies to viral antigens by a radioimmunoprecipitation assay. The results also showed that a Western blot assay for salivary antibodies may be possible. The feasibility of a diagnostic test for human T cell lymphotropic virus type III/lymphadenopathy-associated virus not requiring venipuncture is discussed.

Human T cell lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) is the accepted etiologic agent of acquired immunodeficiency syndrome (AIDS) (5, 9, 16, 18). The routes of transmission of HTLV-III/LAV include close sexual contact, intravenous exposure, and transfusion of blood products (7). Serum antibodies to HTLV-III/LAV have been found in 88 to 100% of AIDS patients and high-risk individuals (4, 17). Antibodies of the secretory immunoglobulin A (IgA) class in mucosal secretions play a protective role in certain viral diseases (14, 15), perhaps as blocking antibodies preventing viral entry into mucosal cells.

Whole saliva of seropositive at-risk individuals contains infectious HTLV-III/LAV (10) and immunoglobulins directed against viral proteins (2). Knowledge of the immune status of mucosal surfaces could be important in studying HTLV-III/LAV pathogenesis. Further, as an alternative to using serum, an accessible fluid such as saliva may prove useful for rapid HTLV-III/LAV antibody testing in populations (such as infants) in which venipuncture is difficult. Other risk groups in which the use of needles is contraindicated, e.g., prison populations, may also benefit from a saliva test.

Whole saliva samples were collected from available at-risk individuals from local hospitals and clinics and also from 10 asymptomatic blood donors seropositive in two enzyme-linked immunosorbent assays (Electro-Nucleonics, Inc., and Abbott Laboratories), in a Western blot assay, and in a radioimmunoprecipitation assay (13); the samples were diluted 1:2 with phosphate-buffered saline with 0.1% sodium azide and centrifuged at 1,000 × g to remove debris. In contrast to a previous report (2), samples were not filtered. Samples of 300-μl of 1:2-diluted saliva were used in both the radioimmunoprecipitation and Western blot assays. Samples were frozen at −20°C until needed. Unprepared samples left at room temperature for as long as 48 h continued to show activity. Patients were categorized by disease in accordance with Centers for Disease Control laboratory and clinical guidelines (8). Their serum samples, obtained at approximately the same time, were tested by an enzyme-linked immunosorbent assay (Electro-Nucleonics) and by radioimmunoprecipitation. As controls, samples were collected from healthy laboratory workers exposed to HTLV-III/LAV and from hospitalized cancer patients. Serum antibody status was compared with salivary antibody status in the same individuals.

Lysates of [35S]cysteine-labeled HTLV-III/LAV-infected and uninfected H9 cells were prepared and incubated with the saliva samples as previously described (2, 6, 12). Samples that radioimmunoprecipitated antigens comigrating with proteins precipitated by a known positive HTLV-III/LAV serum sample were scored as positive. Radioimmunoprecipitation elicited complete concordance between serum and salivary antibody status. Proteins of 160,000 and 120,000 daltons previously shown to be the major HTLV-III/LAV envelope glycoproteins (1, 4, 12) were precipitated by salivary antibodies from all seropositive persons. Antigens of 55,000, 38,000, 27,000, 24,000, and 17,000 daltons were also precipitated by some saliva samples. No seronegative individuals showed detectable HTLV-III/LAV antibodies in their saliva. One individual diagnosed as having AIDS-related complex (ARC) consistently had negative saliva and serum samples. The results are shown in Fig. 1 and Table 1.

For Western blotting, viral antigen-coated nitrocellulose strips were prepared as previously described (3) and incubated with 300-μl saliva samples. To increase antibody detection, we incubated biotin-labeled sheep anti-human immunoglobulin (1:200) with the strips. An antiserum against total immunoglobulin rather than an anti-IgA antiserum was used to maximize the chance of finding virus-specific antibodies in saliva. Of 55 individuals tested by Western blotting (20), all 28 (100%) seropositive individuals showed evidence of salivary antibodies to HTLV-III/LAV antigens. Proteins of 64,000, 55,000, 53,000, 41,000, 34,000, 24,000, and 17,000 daltons were recognized; gp41, p24, and p34 showed the highest-intensity bands. All individuals who tested positive for salivary antibodies by Western blotting were positive for serum and salivary antibodies by radioimmunoprecipitation. The results are summarized in Fig. 2 and Table 2.

We previously reported the detection of antibodies in the saliva of all seropositive at-risk individuals (2), except in some persons with AIDS. In this report, salivary antibodies were detected in all seropositive individuals. Increasing the volume of saliva may have contributed to the improvement

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FIG. 1. Radioimmunoprecipitation assay for salivary antibodies. Soluble cell lysates were prepared from uninfected H9 cells (lanes 1, 3, 5, 7, and 10) and HTLV-III/LAV-infected H9 cells (lanes 2, 4, 6, 8, 9, and 11) exposed to [35S]cysteine, harvested, and lysed with radioimmunoprecipitation buffer. These lysates were then reacted with 300 μl of a 1:2 dilution of saliva which had been bound to protein A beads with 10 μl of sheep anti-human IgA serum. Lanes: 1 and 2, saliva from seropositive blood donor 1; 3 and 4, saliva from seropositive blood donor 2; 5 and 6, saliva from seropositive blood donor 3; 7 and 8, saliva from seropositive blood donor 4; 9, saliva from a cancer patient; 10 and 11, reference serum from an ARC patient known to be positive for HTLV-III/LAV antigens. The sizes of the proteins are given at the left and right in kilodaltons.

of the assay. Antibody proteins may have been bound to the filters, or large antigen-antibody complexes may not have fit through the 0.22-μm pores of the filters used in the previous study.

Two reports detailing the isolation of infectious HTLV-III/LAV from saliva (10, 11) differed greatly in the proportion of virus-positive at-risk individuals found. Filtration was performed on the samples in both studies before incubation with the susceptible cells. Because nonfiltration appears to have increased the detection of antibodies, perhaps deposition of antibody-virus complexes on filters could account for the lower detection of infectious virus.

This study cannot completely address the question of whether antibodies in whole saliva represent a pure secretory IgA immune response. However, failure to incubate an anti-IgA antiserum with the protein A beads resulted in greatly decreased precipitation. Moreover, whole saliva contains approximately 50 times less total antibody than does serum but contains 5 times more IgA than IgG (19). Thus, under normal conditions, most of the immune response in whole saliva is derived from secretory IgA. Transudation of serum into the oral cavity could account for antibody detection in whole saliva. However, all seropositive individuals, including asymptomatic blood donors,

TABLE 1. Comparison of serum and saliva HTLV-III/LAV antibody status determined by radioimmunoprecipitation

<table>
<thead>
<tr>
<th>Health status</th>
<th>No. of persons positive for antibodies to:</th>
<th>gp160 or gp120</th>
<th>p55, p27, p24, or p17</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>Saliva</td>
<td>Serum</td>
</tr>
<tr>
<td>AIDS patients (10)</td>
<td>10</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>ARC patients (13)</td>
<td>12</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Healthy homosexuals (16)</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Cancer patients (6)</td>
<td>NT</td>
<td>0</td>
<td>NT</td>
</tr>
<tr>
<td>HTLV-III/LAV-positive blood donors (10)</td>
<td>10</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Laboratory workers (9)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Healthy sex partners of AIDS or ARC patients (17)</td>
<td>9</td>
<td>9</td>
<td>6</td>
</tr>
</tbody>
</table>

* NT, Not tested.

FIG. 2. Western blot assay for salivary antibodies. Semipurified HTLV-III/LAV as antigen was reacted with saliva samples of the following: seropositive ARC patients (lanes 1, 4, and 5); a seropositive AIDS patient (lane 6); seropositive sexual partners of ARC patients (lanes 2 and 8); a seronegative sexual partner of an ARC patient (lane 9); seropositive healthy homosexual males (lanes 10 and 11); a seronegative healthy homosexual male (lane 3); and a seronegative healthy laboratory worker (lane 7). The sizes of proteins are given at the left in kilodaltons.

TABLE 2. Comparison of serum and saliva HTLV-III/LAV antibody status determined by Western blotting

<table>
<thead>
<tr>
<th>Health status</th>
<th>No. of persons positive for antibodies in:</th>
<th>No. of persons tested</th>
<th>Serum</th>
<th>Saliva</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS patients (4)</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARC patients (10)</td>
<td>9</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy homosexuals (12)</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTLV-III/LAV-positive blood donors (8)</td>
<td>8</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laboratory workers (9)</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy sex partners of AIDS or ARC patients (12)</td>
<td>5</td>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
had detectable salivary antibodies. Patients with AIDS or ARC, more likely to have inflammatory oral conditions, did not demonstrate higher antibody levels. As assayed previously, 6 of 20 patients with AIDS were the only individuals in whom salivary antibodies were not detected (2). This result indicates that oral opportunistic infections were probably not contributing to serum transudation.

All previous HTLV-III/LAV tests have involved the use of serum. The detection of specific antibodies in whole saliva may provide an alternative diagnostic method for individuals in whom venipuncture is contraindicated. Saliva could be used for large-scale viral screening in areas where serum collection might be difficult or economically unfavorable.

Further investigation aimed at developing an easily performed assay for HTLV-III/LAV that uses saliva is warranted. Radioimmunoprecipitation is not a practical assay for situations other than laboratory investigation. Western blotting is more easily performed, although it is time-consuming. This study indicates that other methods of antibody detection, such as enzyme-linked immunosorbent assays, should be researched to develop a test for AIDS that uses saliva.

LITERATURE CITED


