Cervical Antibodies in Patients with Oral Herpes Simplex Virus Type 1 (HSV-1) Infection: Local Anamnestic Responses after Genital HSV-2 Infection

RHODA ASHLEY,1* ANNA WALD,1,2 AND LAWRENCE COREY1,2,3

Departments of Laboratory Medicine,1 Medicine,2 and Microbiology,3 University of Washington School of Medicine, Seattle, Washington 98195

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Herpes simplex virus (HSV)-specific immunoglobulin A, immunoglobulin G, and secretory-component-containing immunoglobulins were identified in cervical and salivary secretions from six subjects with oral HSV type 1 (HSV-1) infections. Anamnestic cervical and salivary antibody responses were detected in two HSV-1-seropositive women with newly acquired genital HSV-2 infections. These data implicate the common mucosal immune system in antibody responses to HSV.

The common mucosal immune system provides local immunity at the site of microbial infection and, in addition, is defined by a redistribution scheme which populates distant mucosae with stimulated lymphocytes (10, 13). If this system includes the genital tract, antibodies to herpes simplex virus type 1 (HSV-1) should be present in genital secretions in response to oral herpes. In turn, these local immune factors might play a role in the partial resistance to the acquisition of HSV-2 genital infections by individuals with prior HSV-1 immunity and in the limitation of the severity of these infections (4–6, 8, 12). A modified Western blot (immunoblot) technique was used to analyze salivary and cervical secretions of eight HSV-1-seropositive women for immunoglobulin A (alpha chain) [IgA(α)], IgG(γ), and secretory-component-containing antibodies (SC-Ig) which bind to HSV proteins.

This study was approved by the University of Washington Human Subjects Review Committee. Subjects 1 through 6 were HSV-1-seropositive immunocompetent adults from whom samples were taken weekly for 4 to 6 weeks. The samples, which were taken from oral and genital mucosae, were tested for both local antibodies and HSV. None of the six subjects had genital herpes; three had oral recurrences, and three remained asymptomatic. Subjects 7 and 8 were enrolled at the University of Washington Virology Research Clinic, with HSV-2 genital infections which were confirmed by culture (15) and by subsequent seroconversion to HSV-2 (3). Both subjects presented on their third day of illness (day 3) with genital lesions, cervicitis, and neck stiffness (subject 7) or urethritis (subject 8). Both began acyclovir (200 mg five times daily for 10 days) on day 3. Specimens for viral isolation were collected from the cervix, vulva, urine, and rectum at enrollment; then at 2- to 4-day intervals until lesions healed at days 11 and 13; and weekly thereafter, for eight weeks. HSV-2 was isolated from multiple sites from both subjects on day 3. On day 5, cervical cultures from subject 7 and cervical and vulvar cultures from subject 8 were positive for HSV-2. Cultures were negative thereafter.

Cervical secretions were sampled at each visit by a previously described wicking technique. Expectorated saliva was centrifuged for 15 min at 12,000 × g. IgA, IgG, and protein concentrations in occult blood-negative secretions were determined (2a). Saliva was diluted 1:10 and cervical secretions were diluted 1:40 before Western blotting with enhanced chemiluminescence was performed for detection of HSV-specific IgA, IgG, and IgM (7) and of SC-Ig with anti-human secretory component (13a). Reactive bands were identified and scored without knowledge of the subjects' HSV status.

Cervical IgA, IgG, and SC-Ig to oral HSV-1. The patients with oral HSV-1 infections, subjects 1 through 6, all had cervical IgG, IgA, and SC-Ig (Table 1). IgA and SC-Ig profiles had fewer bands and were less intense than IgG profiles, despite the finding that total cervical IgA concentrations were

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**TABLE 1. HSV-1 protein-specific IgA, IgG, and SC-Ig in cervical secretions of subjects with oral HSV-1 infections**

<table>
<thead>
<tr>
<th>Subject</th>
<th>HSV-1 target(s)*</th>
<th>Total IgA concn*</th>
<th>Cervical SC-Ig, HSV-1 target(s)</th>
<th>HSV-1 target(s)</th>
<th>Total IgG concn*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>gC</td>
<td>14.8</td>
<td>VP5, gC, gD</td>
<td>VP5, gB, gC, gE, VP16, gD</td>
<td>8.6</td>
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<tr>
<td>2</td>
<td>VP5, gB, gC, gE, gD</td>
<td>17.0</td>
<td>VP5, gB, gC, gE, gD</td>
<td>All 7</td>
<td>14.5</td>
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<tr>
<td>3</td>
<td>VP5, gB, gC, gD</td>
<td>10.8</td>
<td>gB, gC, gD, ICP35</td>
<td>All 7</td>
<td>3.4</td>
</tr>
<tr>
<td>4</td>
<td>gB, gC, gD</td>
<td>7.5</td>
<td>VP5, gB, gC, gD, ICP35</td>
<td>gB, gC, gE, VP16, gD, ICP35</td>
<td>1.2</td>
</tr>
<tr>
<td>5</td>
<td>gC, VP16, gD</td>
<td>13.3</td>
<td>gB, gC, gE, gD</td>
<td>gB, gC, gE, VP16, gD, ICP35</td>
<td>8.4</td>
</tr>
<tr>
<td>6</td>
<td>VP5, gB, gC, gE</td>
<td>28.9</td>
<td>VP5, gD</td>
<td>All 7</td>
<td>6.3</td>
</tr>
</tbody>
</table>

* HSV-1 protein targets were VP5, gB, gC, gE, VP16, gD, and ICP35.

* Corresponding author. Mailing address: University of Washington, Children's Hospital & Medical Center, CH82, 4800 Sand Point Way N.E., Seattle, WA 98105.
higher than total cervical IgG concentrations (Table 1). Cervical IgA and SC-Ig profiles differed. For example, subject 5 had cervical IgA to gC and to VP16 but lacked IgA to gB and to gE; her cervical SC-Ig reacted with gB and with gE but not with gC (Fig. 1). These comparisons suggest that at least two HSV-specific cervical antibody populations are common, one with and one without secretory component.

Salivary IgA, IgG, and SC-Ig to oral HSV-1. Salivary IgG, SC-Ig, and IgA were detected in subjects 1 through 6. As exemplified by subject 5, IgG-specific salivary and cervical profiles were virtually identical for each subject (Fig. 1). While distinct from IgG profiles, salivary and cervical SC-Ig profiles were virtually identical to one another for each subject (Fig. 1), as would be predicted by their presumed origin from lymphocytes of the common mucosal immune system. In contrast, differences in salivary and cervical IgA profiles were common. For example, the cervical IgA profile from subject 5 lacked gB and ICP35, while the salivary IgA profile lacked VP16 (Fig. 1). In subjects 1 through 6, the total salivary IgA concentration was considerably higher than the total cervical IgA concentration (medians, 52.6 and 14.1 μg/100 μl, respectively), while the salivary IgG concentration ranged from 0 to 0.3 μg/100 μl, substantially lower than the cervical IgG concentration (median, 7.4 μg/100 μl).

Anamnestic cervical antibody responses after HSV-2 infection. Subjects 7 and 8 had cervical and salivary IgA and IgG to HSV at their initial sampling, 3 days after onset of their genital symptoms. Both subjects demonstrated marked increases in cervical IgA and IgG to HSV-1 and HSV-2 proteins between days 3 and 5. New bands were identified in samples from subject 7 (Table 2) and increased numbers and intensities of bands were found in samples from subject 8. Further changes in cervical IgA or IgG were not observed thereafter. De novo IgM to HSV-2 was detected in the cervix as well as the saliva of both subjects at day 5. IgM then waned in both sites despite genital recurrences. In contrast to subjects 1 through 6, subjects 7 and 8 had total cervical IgG concentrations that were higher than the concentrations of cervical IgA (Table 2), possibly because of cervicitis and the associated transudation of serum IgG.

Subjects 7 and 8 developed their full HSV-2 cervical IgA and IgG profiles coincident with the cessation of HSV shedding on day 5. Previous studies of seronegative subjects with genital HSV-2 infections have also demonstrated an inverse correlation between cervical antibodies and the presence of HSV (11). We recently found that six HSV-seronegative women required considerably longer (median, 27 days) than subjects 7 and 8 to develop complete cervical IgA and IgG profiles after genital HSV-2 infection (2a). These rapid anamnestic cervical antibody responses appear similar to those seen with serum antibodies (1, 2, 9) and in local compartments in animal models (14). The lesion durations were 11 and 13 days in subjects 7 and 8, respectively, in contrast to 12 to 38 days in the seronegative genital HSV-2 patients.

In summary, we detected antibodies to HSV-1 in salivary and cervical secretions of six HSV-1-seropositive women who lacked clinical and virological evidence of genital HSV-2 infection. Profiles of local IgA and SC-Ig specific to HSV differed, suggesting that two HSV-specific antibody populations develop at mucosal sites, one with and one without a secretory component. Determining whether these differences are related to different epitopes or to different forms of IgA (monomeric and polymeric) or to different epitope-specific responses requires further studies. We also found evidence for rapid anamnestic local responses in two additional women with prior HSV-1 infections. The association between local responses and resolution of the herpetic episodes in these subjects suggests that such information may be of value in identifying the protective mechanisms against mucosal HSV.

### TABLE 2. Cervical IgA and IgG specific to HSV-1 and HSV-2 in HSV-1-seropositive subjects with genital HSV-2 infections

<table>
<thead>
<tr>
<th>Subject</th>
<th>Day</th>
<th>Cervical IgA</th>
<th>Cervical IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HSV-1 target(s)</td>
<td>HSV-2 target(s)</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>None</td>
<td>gB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>All 7</td>
<td>All 7</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>VP5, gB, gD</td>
<td>gB, gC/gE, VP16, gD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VP5, gB</td>
<td>VP5, gB, gG, gC/gE, VP16, gD</td>
</tr>
</tbody>
</table>

*HSV-1 protein targets were VP5, gB, gC, gE, VP16, gD, and ICP35.

*HSV-2 protein targets were VP5, gB, gG, gC/gE, VP16, gD, and ICP35.

*Expressed in micrograms per 100 μl.

*ND, not done.
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REFERENCES