Detection of Herpes Simplex Virus Type 2-Specific Antibody with Glycoprotein G

FRANCIS K. LEE, R. MARIE COLEMAN, LENORE PEREIRA, PAULA D. BAILEY, MARICE TATSUNO, ANDRÉ J. NAHMIA

Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia 30303, and California Department of Public Health, Berkeley, California 94704

Received 25 March 1985/Accepted 3 July 1985

A recently described herpes simplex virus (HSV) type 2 (HSV-2)-specific glycoprotein (gG-2) was purified on an immunoadfinity column prepared with monoclonal antibody. This purified antigen was used in an immunodot enzymatic assay on nitrocellulose paper for the detection of HSV-2 antibodies in human serum. The test was very sensitive in that HSV-2 antibodies were detected in the convalescent sera of 132 of 134 patients with recurrent genital infections in which HSV-2 had been isolated earlier. Antibodies to gG-2 were detected in 17% of sera obtained within 10 days after the onset of a primary HSV infection and in 95% of sera obtained more than 10 days after onset. The specificity of the immunodot assay was demonstrated by testing sera from 245 HSV-seronegative adults, 344 children, 29 nuns, and 13 patients with primary genital HSV-1 infections. None of these 631 sera was reactive with the gG-2 antigen. When compared with a microneutralization test, the immunodot assay was found to be more specific in detecting HSV-2 antibodies. Reproducibility of the gG-2 assay, obtained by retesting 391 sera, was 95%. Thus, this assay has the sensitivity, specificity, and reproducibility necessary for the measurement of HSV-2 antibodies in seroepidemiological studies.

Since the demonstration of two antigenic types of herpes simplex virus (HSV), type 1 (HSV-1) and HSV-2 (7, 14), numerous methods have been devised to differentiate these two closely related viruses. The recent ability to determine restriction endonuclease digestion patterns of herpesvirus DNA (2) and to use monoclonal antibody in various immunological assays (11, 12) has enabled the typing of viruses isolates with consistent accuracy. However, the delineation of HSV-1 and HSV-2 type-specific antibodies in humans, of particular import for definitive seroepidemiological investigation, has remained difficult because of the extensive antigenic cross-reactivity between the two virus types (15).

The development of monoclonal antibodies for the two HSV types has made it possible to isolate specific HSV proteins (1, 3) which could be applied to more specific antibody assays. It was of particular interest to explore the usefulness for type-specific antibody testing of a recently described HSV-2-specific glycoprotein, gG-2 (13). This glycoprotein is specified by a gene mapping in the S component of the genomic DNA of HSV-2 (4, 13). In this report, we describe the evaluation of an HSV-2 type-specific enzyme-linked immunoadfinity serological assay based on affinity-purified gG-2 as antigen.

MATERIALS AND METHODS

Sera. (i) Animals. Hyperimmune rabbit sera to HSV-1 (Shealey and VR3 strain) and HSV-2 (MS strain) were prepared by four biweekly intravenous injections of about 10⁷ PFU of virus into male New Zealand albino rabbits. The sera were collected 2 weeks after the final injection. Convalescent sera obtained previously (8) from 13 rabbits with keratitis induced by ocular inoculation of HSV-1 (VR3 or Shealey strain) and HSV-2 (MS strain) were also included for testing.

(ii) Humans. Sera were obtained from individuals with clinically manifest and culturally proven HSV-1 or HSV-2 infections. Viral isolates from these patients were typed by restriction endonuclease analysis of viral DNA (2) or by direct immunofluorescence with type-specific monoclonal antibodies (11) or by both. A screening enzyme-linked immunosorbent assay (ELISA) with a pool of Triton X-100-extracted antigens from HSV-1- and HSV-2-infected HEp-2 cells (3) detected and quantitated total HSV antibodies in these sera. Primary HSV infections were defined as those in which no preexisting antibody was detected in the acute sera, with HSV antibodies later demonstrated in the convalescent phase. Recurrent cases were defined as those in which the individual had a history of clinically recurrent genital lesions and in which HSV antibodies were detected at the time of the recurrence.

A collection of 344 sera from children between 1 and 10 years of age was tested. The ages were chosen because transplacental antibodies would have been lost by 1 year of age, and an HSV-2 infection between 1 and 10 years would either have been a result of a neonatal infection or an unusual postnatally acquired HSV-2 infection. The 29 sera from nuns, which had been tested earlier with a microneutralization assay (9), were included because HSV-2 infections would be unlikely in this population. In addition, for purposes of evaluating the reproducibility of the assay, 391 sera collected from ongoing epidemiological studies in a health maintenance organization were used.

For standardization of the gG-2 serological assay, 10 convalescent sera from patients with primary HSV-2 infections were pooled and used as HSV-2-positive controls, 20 high-titer sera from patients with HSV-1 infections were pooled and used as HSV-1-positive controls, and 20 sera from healthy donors, negative in the screening ELISA (3), were pooled and used as negative controls.

Antigen preparation. Two mouse monoclonal antibodies, H966 and H1206, were used to prepare immunoaffinity columns for the isolation of gG-2 from HEp-2 cells infected with the G strain of HSV-2. The properties of the H966 antibody and the detailed procedure for the immunoaffinity purification of HSV proteins have been described elsewhere.
Thus, gave gG-2, with analysis. The gG-2 antigen were diluted antigen drying repeating platform wells. The gG-2 antigen preparation was diluted in Tris-buffered saline (TBS; pH 7.2). Optimal dilutions of antigens were determined in block titrations with a known HSV-2-positive serum pool and were 1:16 for the H966 lot and 1:64 for the H1206 lot. Onto the center of each disk, 1 μl of the diluted antigen was delivered with a microsyringe fitted with a repeating dispenser (The Hamilton Co., Reno, Nev.). After drying at a.t. overnight, the disks in the 96-well plates were washed once with distilled water and left to dry completely at ambient temperature (a.t.). The gG-2 antigen preparation was diluted in Tris-buffered saline (TBS; pH 7.2). Optimal dilutions of antigens were determined in block titrations with a known HSV-2-positive serum pool and were 1:16 for the H966 lot and 1:64 for the H1206 lot. Onto the center of each disk, 1 μl of the diluted antigen was delivered with a microsyringe fitted with a repeating dispenser (The Hamilton Co., Reno, Nev.). After drying at a.t. overnight, the disks in the 96-well plates were washed once with TBS (10 min) and incubated at a.t. for 30 min on a rotating platform (TekPro; American Hospital Supply Corp., Evanston, Ill.) with 100 μl of TBS supplemented with 5% bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, Mo.). The buffer was then removed, and 100 μl of test sera, diluted 1:50 in TBS-BSA, was added to duplicate wells. The plates were placed on the rotating platform and left to incubate overnight at a.t. The wells were washed with TBS (three times for 10 min with rotation) and reblocked with 100 μl of TBS-BSA for 30 min. Goat anti-human immunoglobulin G peroxidase conjugate (Miles Laboratories, Inc., Elkhart, Ind.) was diluted in TBS-BSA (1:1,000), and 100 μl was added to each well. Incubation took place at a.t. for 1 h on the rotating platform. After the plates were washed as before, 100 μl of substrate solution (6 mg of 4-chloro-1-naphthol dissolved in 2 ml of methanol mixed with 10 ml of TBS and 5 μl of 30% H2O2) was added to each well. The reaction was stopped after 15 min by removing the substrate solution and washing the plate twice with distilled water. The nitrocellulose disks were read after drying overnight at a.t. in the dark. A positive reaction was indicated by the presence of a bluish-purple dot in the center of the disk. Each assay was read by two individuals; results in the duplicate wells needed to be agreed to by both readers or the test was repeated. The following serum controls were included in each assay: HSV-2-positive serum pool diluted 1:400 and 1:1,600; HSV-1-positive serum pool diluted 1:50 and 1:200, and a negative serum pool diluted 1:50. To ensure standardization of the assay, results were accepted only when both dilutions of the HSV-2 serum pool were positive and all the other controls were negative. No more than 5% of the tests were repeated because of a reading discrepancy, and <1% were repeated because of unacceptable controls.

**Microneutralization test.** The technique of the microneutralization assay for detecting type-specific HSV antibodies has been described previously (8, 9).

### RESULTS

Reactivity of gG2 with rabbit sera. Sera from nine rabbits hyperimmunized with HSV-1 (Shealey or VR3 strain) and convalescent sera from 13 rabbits with HSV-1 keratitis were tested with antigens prepared from the monoclonal H1206 column. None reacted with gG-2 in the assay. In contrast, all six sera from rabbits immunized or infected ocularly with HSV-2 (MS strain) yielded positive reactions. Sera from nonimmunized rabbits or from those hyperimmunized with Hep-2 cells and fetal calf serum were also negative in the assay.

Reactivity of gG2 with human sera. Sensitivity and specificity studies with human sera from 13 primary HSV-1 infections and 14 primary HSV-2 infections demonstrated results identical to those obtained with gG-2 prepared from the H966 and the H1206 columns. The data with human sera obtained by using gG-2 prepared from the H966 column are noted below.

(i) **Sensitivity.** The sensitivity of the assay for HSV-2 antibodies was evaluated by using sera of 134 patients with recurrent genital herpes in which HSV-2 had been isolated (Table 1). Antibody to gG-2 was demonstrated in 99% of sera collected from 134 recurrent cases. When sera obtained from 32 patients with primary HSV-2 infections were tested, antibody to gG-2 was detected in only 17% of sera obtained within 10 days of onset; 95% of sera obtained thereafter were positive (Table 1).

(ii) **Specificity.** The specificity of the assay was evaluated by testing sera from individuals in several special categories (Table 2). Direct evidence of specificity was obtained by demonstrating no reactivity to gG-2 with all 13 of the convalescent sera from patients with primary genital HSV-1 infections. Moreover, none of the 245 sera identified as seronegative to HSV by the screening ELISA (3) reacted with gG-2. Of the 344 sera from children 1 to 10 years of age, 167 reacted with the screening ELISA (3) and two reacted in the gG-2 immunodot enzyme assay. On analysis of the clinical records of these two children, it was found that one serum sample had been included by mistake in that it had been obtained from a 4-month-old infant who had received

### TABLE 1. Sensitivity of immunodot gG-2 antibody assay

<table>
<thead>
<tr>
<th>Patients with HSV-2 isolated</th>
<th>No. of cases</th>
<th>No. (%) with gG-2 antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrent cases</td>
<td>134</td>
<td>132 (99)</td>
</tr>
<tr>
<td>Primary cases (days after onset)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10</td>
<td>12</td>
<td>2 (17)</td>
</tr>
<tr>
<td>11–20</td>
<td>15</td>
<td>14 (93)</td>
</tr>
<tr>
<td>&gt;20</td>
<td>5</td>
<td>5 (100)</td>
</tr>
</tbody>
</table>

### TABLE 2. Specificity of immunodot gG-2 antibody assay

<table>
<thead>
<tr>
<th>Group tested</th>
<th>No. positive/total tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary genital HSV-1</td>
<td>0/13</td>
</tr>
<tr>
<td>Seronegative (screening test)</td>
<td>0/245</td>
</tr>
<tr>
<td>Children</td>
<td>2/344</td>
</tr>
<tr>
<td>Nuns</td>
<td>0/29</td>
</tr>
</tbody>
</table>

* A 4-month-old child had also received gamma globulin earlier, and a 9-year-old girl had vulvar lesions compatible with genital herpes.
gamma globulin a few days earlier. The other serum sample was from a 9-year-old girl who had been hospitalized 2 weeks earlier with vulvar lesions compatible with genital herpes. Of interest is that all 29 sera from nurses were negative by the gG-2 assay, 19 having reacted in the screening ELISA.

As an indirect measure of the specificity of the gG-2 antibody assay, we compared the results of this test with those obtained earlier with a microneutralization assay in 54 sera (9). The results agreed with those observed in all sera previously reported as seronegative or as possessing only HSV-1 antibodies (Table 3). However, 8 of 23 sera previously noted to be positive for type 2 antibody by the microneutralization test were found to be negative by the gG-2 immunodot assay.

(iii) Reproducibility. We selected 291 sera, collected at a health maintenance organization, which were positive for gG-2 antibody. On repeat of the test, 273 (94%) were again found to be positive. When 100 sera collected consecutively at the health maintenance organization were repeated as coded samples, 97 were found to show results (positive or negative) similar to those found in the first testing.

**DISCUSSION**

Since HSV-2 is the type most usually associated with genital or neonatal infections (6), a serological assay that can detect HSV-2 antibodies would be of particular epidemiological assistance. However, because of the existence of many common antigens in HSV-1 and HSV-2 (15), specificity of the assay has been a major problem. Cross-absorption of patient sera with HSV-1 antigens (e.g., reference 10) has limitations because sera with high-titer antibodies to HSV-1 occur frequently and complete absorption of cross-reacting antibody is often very difficult. Moreover, the procedure of cross-absorption may deplete homologous antibodies. Neutralization potency measurements (8) or determinations of HSV-1/HSV-2 ratios in microneutralization or other immunological assays (10, 16) are most accurate in sera that have antibody to either HSV-1 or HSV-2. In patients with both HSV-1 and HSV-2 infections, interpretation of the antibody type(s) can become difficult. For instance, 8 of 23 sera positive for HSV-2 by the microneutralization assay were later found to be negative by the gG-2 assay (Table 3). In view of the high sensitivity of the gG-2 assay (99%; Table 4), we have found that the HSV-2 antibodies detected in the microneutralization test represent false-positive results.

With the availability of mouse monoclonal antibodies, it has become possible to purify HSV-2 proteins that fail to express type-common antigenic determinants detectable in serological assays. The use of gG-2, purified from extracts of HSV-2-infected cells, enabled us to develop an assay of high sensitivity, specificity, and reproducibility. Moreover, the immunodot assay is suitable for screening large numbers of sera because it requires a small amount of purified glycoprotein. Purified gG-2 retains antigenicity at −70°C for over 1 year when stored in glass but not plastic ampoules. We have also noticed that BSA from different sources could influence the results of the gG-2 assay (some batches of BSA caused significant reductions in the sensitivity of the assay). We overcome this problem by testing different batches of BSA from several sources and arranged to have a large stock of the optimal batch from its supplier.

Any serologic assay for HSV-2-specific antibodies requires thorough evaluation because of the epidemiological implications, e.g., the relation of genital herpes with cervical cancer (5, 17). At the individual level, false-positive results might lead to great problems, e.g., improper management for pregnant women or undue psychological trauma in patients and their consorts. The gG-2 serological assay for detecting HSV-2 antibodies has therefore been subjected to assiduous controls for sensitivity, specificity, and reproducibility. The results obtained indicate its usefulness for more definitive measurement of HSV-2 antibodies than has hertofore been available in seroepidemiological studies.

**ACKNOWLEDGMENT**

This work was supported by Public Health Service grant AI-19554 from the National Institute of Allergy and Infectious Diseases.

**LITERATURE CITED**


