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Comparison of Western Blot (Immunoblot) and Glycoprotein G-Specific Immunodot Enzyme Assay for Detecting Antibodies to Herpes Simplex Virus Types 1 and 2 in Human Sera

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Sera from patients with culture-proven genital herpes infections were tested for herpes simplex virus type 1 (HSV-1)- and HSV-2-specific antibodies by both a Western blot (immunoblot) technique (WBA) and immunodot enzyme assays (IEAs) specific for HSV-1 or HSV-2 glycoprotein G (gG). Of 137 serum samples tested, none was mistyped by either WBA or IEA. Both tests were most sensitive with sera obtained at least 21 days after onset of primary HSV-2 infections or sera drawn during recurrent HSV-2 genital episodes: 75 of 76 (99%) such serum samples were positive for HSV-2 antibody by WBA and 73 of 76 (96%) were positive by IEA. Of sera drawn earlier than 21 days from onset of primary genital HSV-2, antibodies to HSV-2 were detected in 25% by WBA and 8% by IEA. In patients with culture-proven primary genital HSV-1 infection, WBA detected antibodies to HSV-1 proteins in 16 of 17 (94%) serum samples drawn at least 21 days after onset of primary genital HSV-1 infection, compared with 9 of 17 (53%) serum samples tested for gG-1 by IEA. Both WBA and IEA are accurate and sensitive tests for HSV-2 antibody in patients convalescing from a first episode or having symptomatic or asymptomatic recurrent genital herpes. WBA was more sensitive than IEA in detecting seroconversion following primary HSV-1 genital herpes, although both assays may miss persons undergoing early seroconversion to HSV-2.

Seroepidemiology studies of herpes simplex virus (HSV) infections have been hampered because of the difficulty in accurately identifying antibodies to HSV type 1 (HSV-1) and HSV-2, especially in persons who have been infected with both viruses (9, 10, 12, 15). Recently, techniques based on antibody reactivity to HSV-1- and HSV-2-specific proteins have been developed that allow accurate serologic typing (1a, 3, 5–7). Glycoprotein G (gG-1 and gG-2) in particular has proven to be a predominantly type-specific target of the human humoral response in both gG-specific immunoassays (5, 6) and Western blot (immunoblot) assays (WBAs; 1a). We report here on the comparative type specificity and sensitivity of immunodot enzyme tests directed at gG-1 and gG-2 (5, 6) and a WBA (1a) directed against a number of HSV-1- or HSV-2-infected cell proteins. The tests were used to detect antibodies to HSV-1 and HSV-2 proteins in sera from patients with culture-proven HSV infections.

MATERIALS AND METHODS

Sera. Sera were obtained from patients enrolled at the Viral Disease Clinic, University of Washington. All patients had HSV-1 or HSV-2 isolated from genital lesions which were sampled at the time that acute-phase serum samples were drawn. Viral isolates were subtyped by restriction enzyme analysis of DNA (11), by indirect immunofluorescence with monoclonal antibodies (11), or by both methods. All acute-phase sera were obtained within 7 days of lesion onset. Convalescent-phase sera were drawn 21 to 40 days after the onset of symptoms. Patients were monitored at monthly intervals thereafter.

Patients were classified on the basis of their clinical histories as having first-episode or recurrent genital herpes. Those who lacked complement-independent neutralizing antibody (4, 10) in their acute-phase sera were defined as having primary first-episode infections. Those with first-episode genital infections who had neutralizing antibody to HSV-1 in their acute-phase sera were classified as having nonprimary first-episode genital herpes (4, 10). This group of patients thus had had HSV-1 infections prior to developing genital HSV-2 infections. These patients had HSV-2 isolated from genital lesions and denied having had prior genital herpes.

Sera from 10 patients who denied having had oral or genital herpes and who were seronegative for complement-independent neutralizing antibodies were also evaluated.

WBA. Antigen was prepared from human embryonic fibroblasts infected with 10^7 50% tissue culture infective doses ($TCID_{50}$) of HSV-1 (strain E115) or HSV-2 (strain 333) per ml. Cells were lysed in 2× SPS (125 mM Tris [pH 6.8], 20% glycerol, 8% sodium dodecyl sulfate, 10% 2-mercaptoethanol, 0.025% bromophenol blue), and 0.25-ml portions were either stored at −70°C or boiled for 2 min before electrophoresis into 9% discontinuous polyacrylamide gels (2). The protein content of the antigen was adjusted to 1.5 mg/ml (Bradford assay; Bio-Rad Laboratories, Richmond, Calif.). After electrophoresis at 20 mA for 90 min, gels were equilibrated for 30 min in transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol, and 0.05% sodium dodecyl sulfate in 25 mM Tris) and then transferred to nitrocellulose (NC) at 200 mA for 2 h. The NC was then cut into 3-mm-wide strips and stored dry at 4°C.

NC strips were blocked with PBS-Tween (0.05% Tween 20 in phosphate-buffered saline [PBS]) and then incubated overnight with 10 μl of human serum diluted 1:100 in Blotto (5% nonfat dry milk, 0.01% thimerosal, 0.01% antifoam A in PBS). NC strips were washed three times for 5 min each with PBS-Tween and then rinsed with PBS. Peroxidase-conjugated anti-human immunoglobulin G (IgG) (Boehringer Mann-

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heim, Indianapolis, Ind.) was diluted 1:1,650 in PBS and incubated with each NC strip for 70 min. Strips were washed three times with PBS-Tween and then rinsed with TBS (Tris-buffered saline; 500 mM NaCl in 20 mM Tris, pH 7.5), and stained with 4-chloro-1-naphthol substrate containing solution A (20 ml of cold methanol and 60 mg of 4-chloro-1-naphthol) and solution B (60 μl of cold 30% H_2O_2 and 100 ml of TBS). After 10 min, strips were rinsed with water and allowed to dry.

Control serum pools were reacted with HSV-1 and HSV-2 antigens in each staining run. Each pool consisted of sera from 10 individuals with culture-confirmed and typed HSV isolates. Each serotype was confirmed by adsorption blot assay (see below). In addition, in each run, an HSV-2 Western blot was reacted with monoclonal antibody AP1 (kindly provided by A. C. Minson, University of Cambridge). This monoclonal antibody reacts primarily with the 92,000-M_r form of gG-2 (8).

Adsorption of sera. Each serum sample to be adsorbed was diluted 1:20 in TST (0.9% NaCl and 0.01% thimerosal in 50 mM Tris, pH 7.5), and 400 μl was added to 1 ml of prepared HSV-1–Sepharose or HSV-2–Sepharose immunosorbent (14). After overnight incubation at 4°C, the Sepharose was pelleted and the supernatants were adjusted to 2 ml in Blotto. Adsorbed sera were then tested on Western blot strips as described above. Postadsorption patterns have been described in detail (3, 14). Briefly, sera from patients with HSV-1 infections contain antibodies which bind preferentially to the HSV-1 ligand. As a result, little or no reactivity is seen on subsequent WBA following HSV-1 adsorption. However, after serum from the same patient is adsorbed against HSV-2 proteins, most reactivity to HSV-1 proteins remains intact. Sera from patients with HSV-2 infections selectively lose reactivity on WBA after adsorption with HSV-2 proteins; HSV-2 reactivity remains detectable after adsorption with HSV-1 proteins. Patients with antibodies to both HSV-1 and HSV-2 have reactive antibodies against HSV-1 (following HSV-2 adsorption) and HSV-2 (following HSV-1 adsorption).

Immunodot enzyme assay. Serum antibodies to HSV-1 gG and HSV-2 gG were detected as described previously (5, 6). Briefly, immunoaffinity column-purified gG-1 (6) and gG-2 (1, 13) were diluted in TBS (pH 7.2), dotted onto nitrocellulose disks, and then placed into 96-well plates. Disks were blocked with 5% bovine serum albumin (BSA) in TBS (TBS-BSA). Test sera, diluted 1:50 in TBS-BSA, were added and incubated overnight at room temperature on a rotating platform. After being washed three times in TBS, disks were reblocked with TBS-BSA and secondary antibody (goat anti-human IgG peroxidase conjugate diluted 1:1,000 in TBS-BSA; Miles Laboratories, Elkhart, Ind.) was added. After 1 h, disks were washed and substrate was added (6 mg of 4-chloro-1-naphthol in 2 ml of methanol mixed with 10 ml of TBS and 5 μl of 30% H_2O_2). Disks were rinsed with distilled water after 15 min and read after drying overnight.

Microneutralization. Complement-independent neutralizing antibody assays were performed as described previously (4, 10).

RESULTS

Determination of HSV serotype by WBA. Sera with only HSV-1 antibodies reacted primarily with the HSV-1 strip and, in addition, lacked antibody to the 92,000-M_r form of gG-2 (gG-92) on the HSV-2 strip (Fig. 1, lanes 1 and 2). Most sera reacted with 11 to 18 HSV-1 proteins and fewer than 6 HSV-2 proteins. HSV-2 bands were faint with most sera.

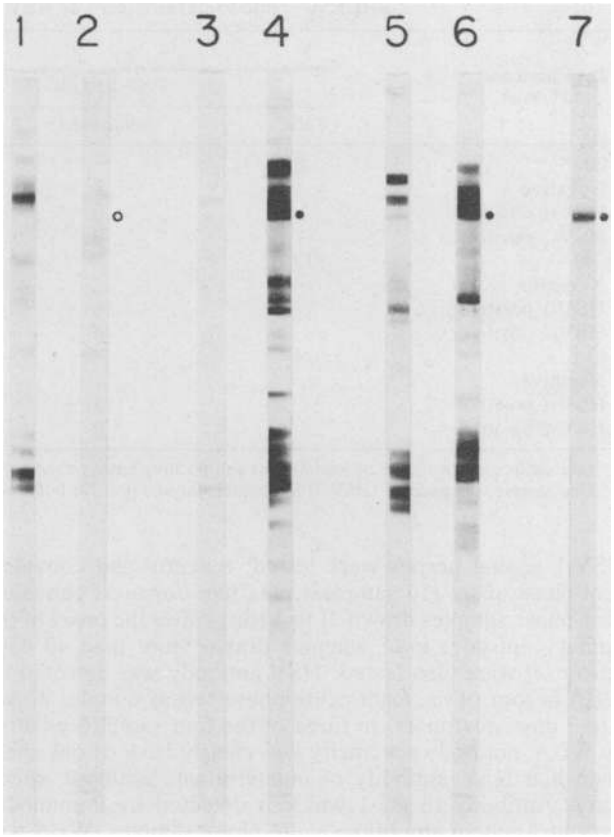

FIG. 1. Specific antibody to HSV-1 and HSV-2 by WBA. Western blots prepared with HSV-1-infected cell lysates (lanes 1, 3, and 5) or HSV-2-infected cell lysates (lanes 2, 4, and 6) were reacted with sera from a patient with primary genital HSV-1 (lanes 1 and 2), a patient with primary genital HSV-2 (lanes 3 and 4), or a patient with active recurrent genital HSV-2 and a history of HSV-1 mucocutaneous infection (lanes 5 and 6). Monoclonal antibody to gG-2 was reacted with an HSV-2-containing NC strip (lane 7) to localize this protein. Note that the gG-2 band appears only on those HSV-2 strips which have been reacted with serum which contains HSV-2 antibodies (solid circles). HSV-1 antibody does not bind to gG-2 (lane 2, open circle).

Some sera, however, had considerable reactivity on the HSV-2 strip and required preadsorption to clarify the HSV-1 subtype. Sera with HSV-2 antibodies reacted primarily with proteins on the HSV-2 strip, including gG-92 (Fig. 1, lanes 3 and 4). These sera reacted with 16 to 24 HSV-2 proteins and fewer than 6 HSV-1 proteins. HSV-1 bands were faint when reacted with sera containing antibodies to HSV-2 only. Sera with both HSV-1 and HSV-2 antibodies had full antibody profiles apparent on both HSV-1 and HSV-2 strips and clear staining of the gG-92 band (Fig. 1, lanes 5 and 6). A positive WBA result was defined as at least four bands which comigrated with those on the positive control strip(s).

Antibody profiles differing from those shown in Fig. 1 (atypical reactivity) occurred with 17 of 137 serum samples. These sera were tested by preadsorbing the sera and then repeating the WBA; 2 were HSV-1; 14 were HSV-2; and 1 had both HSV-1 and HSV-2. Equivalent reactivity without a clear gG-92 marker occurred in 6 of 137 serum samples. Two of these were typed as HSV-2 and four were typed as HSV-1 plus HSV-2.

Sera from patients with HSV-1 infection. Twenty-five serum samples from patients with culture-proven primary

TABLE 1. Time to development of HSV antibodies by immunodot enzyme assay and WBA

Days postonset and result	No. of serum samples					
	HSV-1 patients			HSV-2 patients		
	Total	Immunodot	WBA	Total	Immunodot	WBA
2–20	8			12		
Negative		8	4		11	9
HSV-1 positive		0	3[a]		0	0
HSV-2 positive		0	0		1	3
21–40	13			14		
Negative		7	0		2	1
HSV-1 positive		6	12[b]		0	0
HSV-2 positive		0	0		12	13
>40	4			14		
Negative		1	0		0	0
HSV-1 positive		3	4		0	0
HSV-2 positive		0	0		14	14

[a] One sample was negative by immunodot and positive but type indeterminate by WBA at day 2 postinfection.
[b] One sample was positive (HSV-1) by immunodot and positive but type indeterminate by WBA at 26 days postinfection.

HSV-1 genital herpes were tested: 8 acute- and convalescent-phase pairs (16 samples) plus five unpaired convalescent-phase samples drawn 21 to 40 days after the onset of the primary episode. Four samples drawn more than 40 days postonset were also tested. HSV antibody was detected by WBA in four of the eight acute-phase serum samples drawn 2 to 7 days postonset. In three of the four samples positive by WBA, antibody specificity was clearly HSV-1; one specimen had HSV antibody of indeterminate antibody specificity. Antibody to gG-1 was not detected by immunodot enzyme assay in any of the acute-phase samples. WBA was positive in all 17 of the samples drawn at least 21 days postonset (16 with HSV-1 antibody and 1 with type-indeterminate antibody). Anti-gG-1 antibody was detected by immunodot enzyme assay in 9 of these 17 samples (Table 1). Two (8%) of the 25 samples from HSV-1-infected patients required adsorption for serotyping by WBA. These two samples were collected 21 to 40 days postonset and were negative for antibody to gG-1.

Seroconversion between acute- and convalescent-phase serum pairs was shown by WBA in five of the eight paired specimens and an increase in staining was observed in the other three pairs, indicating a rise in the level of antibody binding to the blots. Seroconversion was apparent in two of eight pairs by immunodot enzyme assay. None of the acute- or convalescent-phase sera was mistyped as HSV-2 by either assay.

Sera from patients with HSV-2 infections. Forty specimens were tested from patients with primary HSV-2 infections. Eleven acute-phase (1 to 6 days postonset) and convalescent-phase (21 to 40 days postonset; median, 26 days) serum pairs (22 total) were tested from patients with culture-proven primary HSV-2 genital herpes. One additional, unpaired acute-phase serum sample and three additional convalescent-phase serum samples were also tested, for a total of 12 acute- and 14 convalescent-phase specimens. Finally, 14 samples drawn more than 40 days postonset were also tested.

Nine of 12 acute-phase serum samples were negative by both tests; 1 was HSV-2 seropositive by both tests and 2 were HSV-2 seropositive by WBA only (Table 1).

Of 14 convalescent-phase samples, 12 had HSV-2 antibody by both tests; 1 was seronegative by both tests (25 days postonset), and 1 was negative by immunodot enzyme assay and HSV-2 seropositive by WBA (32 days postonset). The 14 samples drawn more than 40 days after the primary episode all had only HSV-2 antibody by both tests. Two of the 14 (14%) required adsorption for serotyping. Antibody to HSV-1 was not detected in any of the 40 serum samples from primary genital HSV-2 patients.

Overall, of 27 convalescent-phase serum samples (days 21 to 40 postonset) from patients with primary HSV-1 or HSV-2 infections, serotyping was possible for 25 (93%) by WBA, versus 18 (67%) by immunodot enzyme assay ($P < 0.05$). The major difference in the two tests was the greater sensitivity of the WBA for detecting HSV-1 antibodies within 40 days of the HSV-1 primary episode: 15 of 21 for WBA versus 6 of 21 for immunoblot ($P < 0.01$). In sera drawn within 40 days of HSV-2 primary episodes, 16 of 26 were serotyped by WBA (62%) and 13 of 26 (50%) were serotyped by immunodot enzyme assay (not significant).

Sera from patients with recurrent genital HSV-2. Forty-eight serum samples from patients with HSV-2 culture-proven recurrent genital herpes were also tested (Table 2). The results for 45 of the 48 were the same in both tests: 27 had HSV-2 antibody only and 18 had both HSV-1 and HSV-2. Three samples were typed differently by the two tests: one was seronegative by immunodot enzyme assay and HSV-2 seropositive by WBA and two were HSV-2 positive by immunodot enzyme assay and HSV-1 plus HSV-2 positive by WBA. Sixteen of 48 (33%) samples required adsorption to determine their antibody specificity in the WBA. Of the 16, 11 had HSV-2 antibody by WBA and immunodot enzyme assay, while 5 had HSV-1 and HSV-2 antibody by both tests.

Sera from patients with nonprimary first-episode genital HSV-2. To test the immunodot enzyme assay and WBA for

TABLE 2. Immunodot enzyme assay and WBA results on sera from patients with recurrent genital HSV-2 infection

WBA result	No. of immunodot results				
	Negative	HSV-1	HSV-2	HSV-1 + HSV-2	Total
Negative	0	0	0	0	0
HSV-1	0	0	0	0	0
HSV-2	1	0	27	0	28
HSV-1 + HSV-2	0	0	2	18	20
Total	1	0	29	18	48

TABLE 3. Serology results for patients with culture-proven nonprimary first-episode genital HSV-2 infection[a]

Patient no.	HSV antibody type detected				Days between serum samples
	Acute phase		Convalescent phase		
	WBA	Immunodot	WBA	Immunodot	
1	1	1	1 + 2	1 + 2	20
2	1	1	1 + 2	1 + 2	42
3	1	1	1 + 2	1 + 2	28
4	1	1	1 + 2	1 + 2	23
5	1	1	1 + 2	1 + 2	23
6	1	1	1 + 2	1	30
7	1	1	1 + 2	1	23
8	1	1	1	1	15
9	1	1	1	1	19
10	1 + 2	1	1 + 2	1	15
11	1 + 2	1 + 2	1 + 2	1 + 2	17
12	1 + 2	1 + 2	1 + 2	1 + 2	65

[a] All patients had HSV-1 specificity in acute-phase sera by complement-independent neutralizing antibody and all had HSV-2 isolated from genital lesions.

their ability to detect HSV-2 antibody in the presence of HSV-1 antibody, we tested acute- and convalescent-phase sera from 12 patients who were seen during their first genital herpes episode and who had had prior HSV-1 infections as evidenced by HSV-1 neutralizing antibody in their acute-phase sera (Table 3). All had HSV-2 isolated from genital lesions during this episode. Five of these 12 patients (patients 1 to 5) showed seroconversion to HSV-2 in both tests: their acute-phase sera had HSV-1 antibody and their convalescent-phase sera had both HSV-1 and HSV-2 antibody. Two patients (6 and 7) had antibody to HSV-1 in acute-phase sera by both tests but showed HSV-2 seroconversion by WBA only. Two patients (8 and 9) had HSV-1 antibody only by both tests in both serum samples. One patient (patient 10) had HSV-1 antibody only in both sera by immunodot enzyme assay but had HSV-1 and HSV-2 antibody in both sera by WBA. Finally, immunodot enzyme assay and WBA revealed that two patients (11 and 12) had both HSV-1 and HSV-2 antibody in both acute- and convalescent-phase sera. Three samples (2 from patient 10) and the convalescent-phase serum from patient 2 required adsorption for serotyping.

The median time elapsed between acute- and convalescent-phase samplings was 23 days. The two serum pairs for which HSV-2 seroconversion was not documented by either test were drawn 15 and 19 days apart. The three additional pairs which had only HSV-1 antibody by immunodot enzyme assay were drawn 15, 23, and 30 days apart.

Sera from patients without HSV neutralizing antibody. Ten serum samples which were seronegative by complement-independent neutralizing assay were tested by immunodot enzyme assay and WBA. Seven of these samples (70%) were also seronegative by both immunodot enzyme assay and WBA. Two of 10 were seropositive by immunodot enzyme assay (HSV-1 or dual antibody) but seronegative by WBA, and one sample was HSV-1 seropositive by WBA but seronegative by immunodot enzyme assay.

Concordance between immunodot enzyme assay and WBA tests. Of 147 serum samples tested, concordant results were obtained for 122 (83%). Of the 25 samples for which different results were obtained, 15 (60%) were immunodot enzyme assay negative and WBA positive, 11 for HSV-1 and 4 for HSV-2. In 6 of the 25 discordant samples, WBA was positive for both HSV-1 and HSV-2, while immunodot enzyme assay was positive for only HSV-1 ($n = 4$) or HSV-2 ($n = 2$). The immunodot enzyme assay typed two samples as HSV-1 that were untypeable by WBA. Two other samples were seronegative by WBA but positive for either HSV-1 or dual antibody by immunodot enzyme assay.

DISCUSSION

This study provides evidence for the excellent ability of the WBA and immunodot enzyme assay to type antibodies which arise after genital HSV infections. Of 65 serum samples from patients with primary genital HSV-1 infection ($n = 25$) or primary genital HSV-2 infection ($n = 40$), none was mistyped by either immunodot enzyme assay or WBA. Both tests showed the best sensitivity with sera obtained at least 21 days after the onset of first episodes of symptomatic HSV-2 genital infection or sera from patients with recurrent genital HSV-2. In these settings, WBA detected HSV-2 antibody in 82 of 83 (99%) serum specimens and immunodot enzyme assay detected it in 78 of 83 (94%) serum specimens. Moreover, the concordance between the two assays for these sera was 93%.

Seroconversion to HSV-2 was studied in 12 patients presenting with first-episode genital lesions. These 12 patients had neutralizing antibody to HSV-1 in their acute-phase sera. Two patients failed to seroconvert to HSV-2 by WBA or immunodot enzyme assay by day 15 and 19, respectively. These results reflect the limited sensitivity of these tests for early seroconversion. Conversely, two acute-phase serum samples were positive by both tests for both HSV-1 and HSV-2. These could represent either early detection of seroconversion or unrecognized recurrent disease (and false-negative neutralizing antibody results). It is interesting that both samples from patient 10 required adsorption because no antibody to gG-2 could be detected. The immunodot enzyme assay result confirmed that antibody to gG-2 had not developed in either serum. These patients represent an important challenge for accurate testing by WBA due to the anamnestic response to HSV-1 proteins during HSV-2 seroconversion (1a). Further studies with more patients and longer follow-up appear warranted.

The immunodot enzyme assay and WBA appeared to be more sensitive than an assay for neutralizing antibody. WBA, in particular, detected antibody in half (4 of 8) of the acute-phase sera from primary HSV-1 cases and 25% (3 of 12) of the acute-phase sera from primary HSV-2 cases. By definition, all of these sera were negative by neutralizing assay. These patients had no history of genital herpes, and

only one had developed antibody to gG. Ten samples were tested from patients without history of HSV, without HSV neutralizing antibody, and without signs, symptoms, or cultures positive for HSV. One patient was HSV-1 seropositive by WBA and two were seropositive for antibody to gG-1 or gG-2. These sera either gave false-positive results or represent low-titer antibodies from unrecognized infections. Since the false-positive rate was zero for heterotypic antibodies from 65 samples from true primary cases, it seems unlikely that these three samples lacked HSV antibody. It is possible that the two samples which had detectable antibody to gG were from patients with remote, subclinical infections. The serum positive by WBA is more likely to represent an early seroconversion since antibodies to a number of HSV proteins were present.

The time required to seroconvert to gG-1 or gG-2 following primary infection varied among patients. Antibody to gG-1 was not detected before 21 days in sera from patients with primary genital HSV-1, and only about half of the sera drawn 21 to 40 days after onset of lesions had detectable gG-1 antibody. We also found that antibody to gG-2 was detected earlier than antibody to gG-1.

WBA, which detects antibodies to more than one viral protein, had increased sensitivity for detecting HSV antibodies early in the course of primary infection. Fifteen of 21 (71%) serum samples drawn within 40 days of onset of primary HSV-1 infections were seropositive by WBA, compared with 6 of 21 (29%) by gG-1-specific assay ($P < 0.001$, Fisher's exact test). The two assays had similar sensitivity with sera drawn within 40 days of HSV-2 infection: 16 of 26 (62%) and 13 of 26 (50%) were positive by WBA and immunoblot, respectively.

Sensitivity of the WBA may be improved for low-titer specimens by increasing the volume of serum. We currently perform a second WBA on all sera which yield atypical profiles or fewer than four bands and have found this to be an effective means of detecting seroconversion earlier. Increased sensitivity, however, may be gained at the expense of typing ability, particularly for specimens obtained soon after HSV-2 infection. Because the reading of the WBA for HSV-2 is dependent, in part, on gG-2, sera from patients who have not yet seroconverted to gG-2 can be difficult to distinguish from sera with only HSV-1 antibody (1a).

The dependence of WBA interpretation on gG-2 requires an accurate identification of this band on test strips. Reacting monoclonal antibodies to gG with control strips can distinguish the 92,000-dalton gG-2 band (gG-92) from proteins such as gB, which have similar migration characteristics. In addition, many human sera recognize another species of gG-2 of 70,000 M_r (16). This gG species does not comigrate with other HSV-1 or HSV-2 proteins and therefore provides a clear marker for HSV-2 antibody. However, antibody to gG-70 appears to arise no earlier than antibody to the gG-92 species. When the gG-2 species are not clear on the WBA, sera require repeat testing after cross-adsorption, a procedure which adds time and additional cost to the assay method. Identification of additional HSV-2-specific markers would be useful for those sera which lack antibody to gG-2.

Both assays indicated that about 40% of persons with culture-proven recurrent genital HSV-2 also had HSV-1 antibodies. Patients with first-episode genital HSV-2 and prior HSV-1 antibody tend to mount an anamnestic response to the type-common antigens, with type-specific HSV-2 antibodies arising later (9, 15). This was demonstrated by the fact that HSV-1 reactivity remained stronger than HSV-2 reactivity in 5 of the 18 dual-antibody serum samples from patients with recurrent HSV-2 episodes. These sera required adsorption for serotyping because of the disproportionate HSV-1 staining.

The increased sensitivity and specificity of these tests for HSV-2 antibody now allow accurate seroepidemiologic studies. The immunodot enzyme assay provides a rapid, reproducible method for testing large numbers of sera. Use of the miniblot format for gel electrophoresis and protein transfer allows large-scale testing by WBA, as well. Over 800 strips can be prepared from the antigen derived from a single roller bottle of infected cells. These strips can be prepared in 1 day and are virtually identical in their binding characteristics. The ability of these tests to type HSV antibodies means that unrecognized herpes infections in asymptomatic, history-negative individuals can now be reliably detected. Serologic examination of these people is important for our understanding of HSV pathogenesis.

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