JOURNAL OF CLINICAL MICROBIOLOGY, May 1992, p. 1312–1314
0095-1137/92/051312-03$02.00/0

Vol. 30, No. 5

Comparison of the Western Immunoblot Assay and a Glycoprotein G Enzyme Immunoassay for Detection of Serum Antibodies to Herpes Simplex Virus Type 2 in Patients with AIDS

SHARON SAFRIN,[1,2]* ANN ARVIN,[3] JOHN MILLS,[1,4] AND RHODA ASHLEY[5]

Departments of Medicine,[1] Epidemiology and Biostatistics,[2] Microbiology, and Laboratory Medicine,[4] University of California, San Francisco, California 94143; Department of Laboratory Medicine, University of Washington, Seattle, Washington 98195[5]; and Department of Pediatrics, Stanford University School of Medicine, Stanford, California 94305[3]

Received 25 November 1991/Accepted 3 February 1992

Herpes simplex virus type 2 (HSV-2) seroprevalence in 68 patients with AIDS was 77% by Western blot (immunoblot) and 44% by glycoprotein G-2 immunoassay. Each of 16 patients with culture-proven HSV-2 infection was positive by Western blot versus 8 by glycoprotein G-2 immunoassay. No differences in age, race, duration of AIDS, acyclovir usage, or HSV-1 seroprevalence were found to explain differences in sensitivity.

In recent years, serologic techniques based upon antibody reactivity to herpes simplex virus (HSV)-specific proteins have been useful for assessment of the seroprevalence of HSV-1 and HSV-2 infections (2, 3, 6, 8–10, 14, 15) with either glycoprotein G (gG)-specific immunoassays (3, 6, 8–10, 15) or Western blot (immunoblot) assays (2, 14). Although comparison of an immunodot enzyme assay with a Western blot assay showed comparable sensitivities for immunocompetent individuals (1), the two methodologies have not been compared in patients infected with human immunodeficiency virus (HIV).

We studied 68 HIV-infected patients (67 men and 1 woman) with a histologically documented (12) first episode of *Pneumocystis carinii* pneumonia diagnosed at San Francisco General Hospital in 1986. Clinical characteristics of the patients over 739 patient-months of follow-up were derived from a review of the medical records and have been described in a separate report (13). Virus was typed after routine culture (4) by using fluorescein-conjugated monoclonal antibodies specific for HSV-1 and HSV-2 (Syva, Palo Alto, Calif.). Sera collected at the time of acute *P. carinii* pneumonia were tested for HSV-2 antibodies by Western blot assay (1) and a solid-phase gG-2 enzyme immunoassay (15) by researchers in two different laboratories; each was blind to the other's results and to clinical data. Testing for serum antibody to HSV-1 was performed by Western blotting (1). Continuous data were compared by using the Mann-Whitney test; categorical variables were compared by a two-tailed Fisher exact test.

Of 68 patients, 29 (42.6%) were positive for HSV-2 antibody by both assays (Table 1). The Western blot assay detected HSV-2 antibody in the sera of 52 (77%) of the patients; the gG-2 immunoassay detected HSV-2 antibody in 30 (44%) of the patients. A gG-2 band was visualized in profiles from all but one of the 23 patients who had detectable HSV-2 antibody by Western blotting but not by gG-2 immunoassay (Table 2). No characteristic pattern or differences in band intensity were discernible in patients with results that were discordant by the two assays. Only one patient was seronegative for HSV-2 by Western blotting and positive by gG-2 immunoassay. This patient had antibodies reactive with HSV-2 gB, gC, VP16, and gD but none reactive with VP5, gG, gE, or ICP35, a pattern consistent with HSV-1 seropositivity.

Comparison of the demographic and clinical features of the patients who were HSV-2 seropositive by both tests with those who had discordant HSV-2 serologic results revealed no significant differences in age, race, or duration of AIDS prior to serum testing. Also, history of treatment with acyclovir (47.4 versus 58.8%), history of suppressive acyclovir usage (40 versus 60.5%), and seropositivity for HSV-1 antibody (56.3 versus 52.4%) were not significantly different ($P = 0.6$, 0.2, and 1.0, respectively). The median frequency of HSV recurrence was lower in patients with concordant HSV-2 test results than in those with discordant results but not significantly so (once every 30 versus once every 15 weeks; $P > 0.2$).

Of 68 patients, 16 had culture-documented outbreaks of HSV-2; of these, 8 had HSV-2 antibody detected in serum by Western blot but not gG-2 immunoassay (Table 2). Four patients had culture-proven HSV-2 infection a median of 6 (range, 3 to 215) weeks prior to the date of serum antibody testing; two were seronegative by gG-2 immunoassay. Twelve patients had culture-proven HSV-2 infection 1 to 17 (median, 4) weeks after the date of serum collection; six were seronegative by gG-2 immunoassay (Table 2).

Type-specific assays based on specific antibody reactivity to native gG-2 glycoprotein have been widely used in recent serosurveys evaluating the prevalence of HSV-2 infection (2, 3, 6, 8–10, 14, 15). The Western blot assay for HSV-2 antibodies was used to assess HSV-2 seroprevalence in homosexual men (14) and in immunocompetent patients with first-episode genital HSV (2). The HSV gG-2 immunodot enzyme assay (11) has been used to analyze seroprevalence in women attending family planning clinics (3), university students (6), homosexual men (8, 10), and the general population of the United States (9). The HSV-2 gG immunoassay used in the current study was of value in assessing seroprevalence and risk of vertical transmission in pregnant women (15). Direct comparison of the Western blot method and the gG-2 immunodot enzyme assay of Lee et al. revealed comparable sensitivities in immunocompetent patients with culture-proven recurrent herpes, as well as in those from

* Corresponding author.

TABLE 1. Comparison of Western blot and gG-2 immunoassay detection of HSV-2 antibody in the sera of 68 patients with AIDS

Western blot result	No. with the following gG-2 immunoassay result:		Total (%)
	Positive	Negative	
Positive	29	23	52 (77)
Negative	1	15	16 (23)
Total (%)	30 (44)	38 (56)	68 (100)

whom sera were obtained at least 21 days after the onset of lesions (1). However, the Western blot was more sensitive with respect to detection of HSV antibodies early in the course of primary infection. Our estimate of 77% HSV-2 seroprevalence by Western blot is similar to a previous estimate of 68% in HIV-infected men which was done with an HSV gG-2 immunodot enzyme assay (8). Thus, it is unlikely that the lower sensitivity of the gG-2 immunoassay was due to failure to detect early seroconversion.

Our population was unique in that sera were obtained from patients with clinical AIDS at the time of an acute opportunistic infection, i.e., *P. carinii* pneumonia. Conceivably, an inhibitory factor present in sera from these patients blocked gG antibody detection under gG-2 immunoassay conditions. Alternatively, gG-2 antibody may selectively decrease with the progressive immunosuppression induced by HIV infection. Prospective studies using quantitative antibody measurements and clinical correlation are needed to explore this possibility. In addition, comparison of the seroprevalence of HSV-2 antibody in HIV-infected patients with AIDS, asymptomatic HIV-infected individuals, and HIV-negative persons would be useful in further increasing our understanding of the phenomenon.

TABLE 2. Patients in whose sera antibody to HSV-2 was detected by Western blot but not by gG-2 immunoassay

Patient no.	Antibody to HSV protein by Western blot								Positive HSV culture[a]	History of genital herpes[b]
	VP5	gB	gG	gC	gE	VP16	gD	ICP35		
2	+	+	+	+	+	+	−	+	After	Yes
3	+	+	+	+	+	+	+	+	No	Yes
4	+	+	+	+	+	+	+	+	No	Yes
5	−	+	+	+	+	+	+	+	No	Yes
9	−	+	+	+	+	+	+	−	No	No
10	+	+	+	+	+	+	+	+	After	Yes
13	+	+	+	+	+	+	+	+	No	Yes
21	+	+	+	+	+	+	+	+	Before	Yes
25	+	+	−	+	+	+	+	+	No	Yes
26	+	+	+	+	+	+	−	+	No	No
27	+	+	+	+	+	+	+	+	After	Yes
28	+	+	+	+	+	+	+	+	No	Yes
29	+	+	+	+	+	+	+	+	After	Yes
33	+	+	+	+	+	+	+	+	After	Yes
37	+	+	+	+	+	+	+	+	No	No
38	+	+	+	+	+	+	+	+	No	Yes
39	+	+	+	+	+	+	+	+	Before	Yes
42	+	+	+	+	+	+	+	+	No	No
44	+	+	+	+	+	+	+	+	No	No
59	+	+	+	+	+	+	+	+	No	No
61	+	+	+	+	+	+	+	+	No	No
62	+	+	+	+	+	+	+	+	No	Yes
67	+	+	+	+	+	+	+	+	After	Yes

[a] Culture documentation of HSV infection occurred before or after serum was obtained for testing. In a number of patients, cultures were not performed (no).

[b] History of genital herpes at any time during follow-up or at initial evaluation of patients.

We were unable to detect patient-related factors in those with discordant serologic results to explain the differences in sensitivity of the assays of HSV-2 serum antibodies. In particular, given the decreased rates of seropositivity for HSV-2 serum antibody in HSV-1-seropositive persons reported previously (3), it is of interest that HSV-1 seropositivity did not differ significantly in the concordant and discordant subgroups in our study. Also, since previous studies have shown decreased titers of antibodies to HSV in patients receiving acyclovir chemosuppression (5, 7), we sought an association of chronic or prior acyclovir usage with presence or absence of serum HSV-2 antibody by the two assays. No such association was found, perhaps because of the relatively small number of patients involved.

The HSV gG-2 immunoassay has the potential to provide a rapid, reproducible method for testing large numbers of sera. In comparison with the Western blot technique, it is less labor intensive and less dependent upon technician expertise in the clinical virology laboratory. However, our experience suggests that the sensitivities of these assays are not equivalent for severely immunocompromised individuals. Western blotting may therefore be the preferred assay for detection of HSV-2 antibodies in serosurveys of patients with AIDS or for diagnosis of latent HSV-2 infection in patients with AIDS lacking culture confirmation of infection.

We thank Paul Cusick and Christine Houlihan for assistance with collection of data.

REFERENCES

1. **Ashley, R. L., J. Militoni, F. Lee, A. Nahmias, and L. Corey.** 1988. 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. J. Clin. Microbiol. **26**:662–667.
2. **Bernstein, D. I., M. A. Lovett, and Y. J. Bryson.** 1984. Serologic analysis of first-episode nonprimary genital herpes simplex virus infection. Am. J. Med. **77**:1055–1060.
3. **Brenig, M. K., L. A. Kingsley, J. A. Armstrong, D. J. Freeman, and M. Ho.** 1990. Epidemiology of genital herpes in Pittsburgh: serologic, sexual, and racial correlates of apparent and inapparent herpes simplex infections. J. Infect. Dis. **162**:299–305.
4. **Drew, W. L., and W. E. Rawls.** 1985. Herpes simplex viruses, p. 705–710. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
5. **Erlich, K. S., L. Hauer, and J. Mills.** 1988. Effects of long-term acyclovir chemosuppression on serum IgG antibody to herpes simplex virus. J. Med. Virol. **26**:33–39.
6. **Gibson, J. J., C. A. Hornung, G. R. Alexander, F. K. Lee, W. A. Potts, and A. J. Nahmias.** 1990. A cross-sectional study of herpes simplex types 1 and 2 in college students: occurrence and determinants of infection. J. Infect. Dis. **162**:306–312.
7. **Gold, D., R. Ashley, G. Solberg, H. Abbo, and L. Corey.** 1988. Chronic-dose acyclovir to suppress frequently recurring genital herpes simplex virus infection: effect on antibody response to herpes simplex virus type 2 proteins. J. Infect. Dis. **158**:1227–1234.
8. **Holmberg, S. D., J. A. Stewart, A. R. Gerber, R. H. Byers, F. K. Lee, P. M. O'Malley, and A. J. Nahmias.** 1988. Prior herpes simplex virus type 2 infection as a risk factor for HIV infection. JAMA **259**:1048–1050.
9. **Johnson, R. E., A. J. Nahmias, L. S. Magder, F. K. Lee, C. A. Brooks, and C. B. Snowden.** 1989. A seroepidemiologic survey of the prevalence of herpes simplex virus type 2 infection in the United States. N. Engl. J. Med. **321**:7–12.
10. **Kingsley, L. A., J. Armstrong, A. Rahman, M. Ho, and C. R.**

Rinaldo. 1990. No association between herpes simplex virus type-2 seropositivity or anogenital lesions and HIV seroconversion among homosexual men. J. Acquired Immune Defic. Syndr. **3**:773–779.

11. **Lee, F. K., R. K. Coleman, L. Pereria, P. D. Bailey, M. Tatsuno, and A. L. Nahmias.** 1985. Detection of herpes simplex virus type 2-specific antibody with glycoprotein G. J. Clin. Microbiol. **22**:641–644.
12. **Ng, V. L., I. Gartner, L. A. Weymouth, C. D. Goodman, P. C. Hopewell, and W. K. Hadley.** 1989. The use of mucolysed sputum for the identification of pulmonary pathogens associated with human immunodeficiency virus infection. Arch. Pathol. Lab. Med. **113**:488–493.
13. **Safrin, S., R. Ashley, C. Houlihan, P. S. Cusick, and J. Mills.** 1991. Clinical and serologic features of herpes simplex virus infection in patients with the acquired immunodeficiency syndrome. AIDS **5**:1107–1110.
14. **Stamm, W. E., H. H. Handsfield, A. M. Rompalo, R. L. Ashley, P. L. Roberts, and L. Corey.** 1988. The association between genital ulcer disease and acquisition of HIV infection in homosexual men. JAMA **260**:1429–1433.
15. **Sullender, W. M., L. L. Yasukawa, M. Schwartz, L. Pereira, P. A. Hensleigh, C. G. Prober, and A. M. Arvin.** 1988. Type-specific antibodies to herpes simplex virus type 2 (HSV-2) glycoprotein G in pregnant women, infants exposed to maternal HSV-2 infection at delivery, and infants with neonatal herpes. J. Infect. Dis. **157**:164–170.