JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1993, p. 3157–3164
0095-1137/93/123157-08$02.00/0

Vol. 31, No. 12

Detection of Immunoglobulin M Antibodies to Glycoprotein G-2 by Western Blot (Immunoblot) for Diagnosis of Initial Herpes Simplex Virus Type 2 Genital Infections

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Received 15 April 1993/Returned for modification 6 July 1993/Accepted 31 August 1993

Western blots (immunoblots) for the detection of immunoglobulin M (IgM) antibodies specific for herpes simplex virus type 1 (HSV-1) and HSV-2 in patients' sera were developed. The locations of the type-specific glycoprotein G (gpG-2) of HSV-2 (92- and 140-kDa forms) and glycoprotein C of HSV-1 (gpC-1), which carries mostly type-specific antigenic epitopes, were checked with specific monoclonal antibodies. Western blot assays for IgM antibody to gpC-1 or gpG-2 were performed after depletion of IgG by precipitation with anti-human IgG. In patients with primary HSV-2 genital infections, seroconversion of IgM and IgG antibodies to both the 92- and 140-kDa forms of gpG-2 was observed, although both antibodies appeared in convalescent-phase serum after the first week. IgM and IgG antibodies to low-molecular-size polypeptides (40 to 65 kDa) were the first antibodies observed in patients with primary infection, but these antibodies were cross-reactive with HSV-1 and HSV-2. However, in patients with recurrent HSV-2 infections, IgG antibodies to both forms of gpG-2 and the low-molecular-size polypeptides were found no matter how early after onset the patient was bled, and IgM to gpG-2 did not appear. In patients with nonprimary initial genital HSV-2 infections, IgG antibody to HSV-1 was demonstrated in the first serum specimen, and HSV-2-specific IgM was found in 39% of the serum specimens. Hence, the Western blot assay can be used to test for IgM antibody to gpG-2, allowing for the retrospective diagnosis of initial HSV-2 infections and its use as a supplementary test to the gpG-2 IgG enzyme-linked immunosorbent assays developed elsewhere. In contrast, IgM antibody to gpG-2 is not usually detected in patients with recurrent HSV-2 infections.

Herpes simplex virus type 2 (HSV-2) is the predominant cause of genital herpes, accounting for approximately 85% of cases of primary genital herpes and >95% of cases of recurrent genital herpes in Sydney, Australia, and elsewhere (12; unpublished data). Virus isolation or antigen detection from vesicular or ulcerative lesions is the preferred diagnostic method, but isolation or detection may be difficult if lesions are crusted, healed, or absent. Recently, it has become apparent that subclinical infection is more common than clinically recognized disease (39).

Because many of the polypeptides of both HSV-1 and HSV-2 share common antigenic epitopes, extensive cross-reactivity occurs between antibodies elicited by the two types of virus. This means that all commercial diagnostic test kits currently available for the separate determination of HSV-1 and HSV-2 antibody levels are unreliable because they use whole virus antigens or HSV glycoproteins which detect type "common" antibody (4, 15). There is a need for accurate, routine, and readily available serologic tests for HSV-2-specific antibody. Because asymptomatic HSV-2 infection is common, such tests are necessary for the determination of the communitywide prevalence of HSV-2 infection. They could also have clinical application in the retrospective diagnosis of primary genital herpes, in the assessment of patients reporting recurrent genital lesions, in the identification of HSV-2 infections in sexual partners, and as a screening test for asymptomatic pregnant women in the investigation of strategies to control neonatal herpes (16, 26).

Western blot assay (WBA) has been used for the characterization of HSV-1 and HSV-2 type-specific glycoproteins, according to their apparent molecular sizes, with murine-specific monoclonal antibodies (2, 17, 30, 32, 35, 40). Sensitive and specific enzyme assays which use glycoprotein G (gpG) from HSV-2 (gpG-2) have recently been described for the measurement of HSV-2 immunoglobulin M (IgM) antibodies (20). In our experience, a diagnosis of initial HSV-2 infection may occasionally depend entirely on gpG-2 IgM serology. Supplementary tests such as WBA are required to confirm positive or equivocal enzyme-linked immunosorbent assay (ELISA) results, a role similar to that in human immunodeficiency virus and hepatitis C virus serology. WBA might also define further type-specific epitopes for use in future immunoassays. The slower and more cumbersome WBA has been reported to be more sensitive in identifying seroconversion to HSV-2, especially in patients with prior HSV-1 antibody (7).

The aims of the present study were to initially localize the HSV-1- and HSV-2-specific glycoproteins on Western blots in our own laboratory and compare them with those found in previous reports (7) and then to define the specific IgM and IgG antibody responses to HSV-2-specific glycoproteins during primary, initial nonprimary, and recurrent HSV-2 genital infections. There was found to be no need for cross-adsorption (5, 8) to remove heterotypic and type-common antibodies so that type-specific antibodies could be clearly detected. To our knowledge this is the first report of

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a WBA which can also detect specific IgM as well as specific IgG to HSV-2 gpG-2.

MATERIALS AND METHODS

Human sera. Serum specimens were obtained from patients with primary genital herpes, initial nonprimary genital herpes, and a history of recurrent genital herpes who were attending the Sydney or Parramatta sexually transmissible disease (STD) clinics or consulting medical practitioners mainly in the western area of Sydney, Australia. Informed consent was obtained in all cases.

All patients' sera were tested for total antibodies to HSV by a microtiter complement-fixing antibody test (18) or indirect immunofluorescence antibody test (20), and all isolates from genital lesions were typed by direct immunofluorescence with type-specific monoclonal antibodies by using the MicroTrak HSV-1/HSV-2 identification and typing test kit (Syva Co., Palo Alto, Calif.).

Individuals with *primary genital infections* were defined as (i) those in which the acute-phase serum samples had no preexisting HSV antibodies by the complement-fixing test or indirect immunofluorescent antibody test at the initial clinic visit but who developed a fourfold or greater rise in antibody titer in convalescent-phase serum samples, and (ii) those from whom HSV-2 was isolated from genital lesions. Paired serum specimens were obtained from 17 patients with primary genital HSV-2 infections, and from two of these patients, two additional serum specimens were obtained. Individuals with *initial nonprimary HSV-2 genital infections* (also termed *first-episode genital herpes*) were defined as those who had complement-fixing antibodies to HSV (later shown to be antibody to HSV-1 only by WBA) in the initial serum specimen collected within 2 to 10 days of onset of symptoms. At the same time, swabs from the first genital lesion were collected and HSV-2 was isolated (8). Additional serum specimens were unavailable. These patients had no recollection of having had an earlier herpes-like genital lesion. Twenty-three single serum samples were obtained from 23 patients with recent nonprimary initial HSV-2 genital infections. Individuals with *recurrent HSV-2 genital infections* were defined as those who had a history of previous HSV-2 genital infections and complement-fixing antibody to HSV in acute-phase sera (taken within 2 to 10 days of onset of symptoms). None of the patients in the present study had a significant rise in antibody titer in their convalescent-phase sera. Recurrence was confirmed by isolation of HSV-2. Paired (acute- and convalescent-phase) sera were collected from 12 patients, and an additional serum specimen was collected at 30 to 45 days after the onset of symptoms from each of two patients with recurrent HSV-2 genital infections.

Three paired serum specimens were obtained from patients with primary HSV-1 infection, proven by HSV-1 isolation and with no preexisting antibodies to HSV in the acute-phase sera (but present in the convalescent-phase sera).

Sera randomly selected from 25 children between 1 and 9 years of age, 45 healthy adult Westmead Hospital laboratory staff with no known history of genital herpes, and 30 STD clinic attendees were analyzed by the WBA.

In general, the acute-phase serum samples were drawn at 2 to 10 days after the onset of symptoms, and the convalescent-phase serum samples were drawn at 14 to 28 days after the onset of symptoms. Sequential serum specimens from two patients with proven primary HSV-2 genital infections were drawn at 2 to 7 days after the onset of symptoms at the first clinic visit and at intervals of 12 to 24 days, 30 to 48 days, and 70 to 80 days after the onset of symptoms. Also, sequential serum specimens from two patients with proven recurrent HSV-2 genital infection were drawn at 2 to 7 days after the onset of symptoms at the first visit and at 14 to 28 days and after 30 to 45 days.

Control and reference sera. Serum pools prepared from patients with proven genital HSV-2 infections and IgG or IgM antibody to gpG-2 by ELISA and also from patients who were IgG or IgM seronegative to gpG-2 (20) were used as controls. To characterize the HSV-1- and HSV-2-specific glycoproteins, a murine-specific monoclonal antibody to HSV-1 known to recognize the 130-kDa gpC (gpC-1) and a murine-specific monoclonal antibody to HSV-2 known to recognize the 92-kDa gpG (gpG-2) (kindly provided by S. Jeansson, University of Göteborg, Göteborg, Sweden) were used. A hyperimmune rabbit antiserum to HSV-1 (Dakopatts, Copenhagen, Denmark), a hyperimmune rabbit antiserum to HSV-2 (Dakopatts), and a normal rabbit serum (Dakopatts) were reacted against both HSV-1- and HSV-2-infected and uninfected cell lysates.

WBA. (i) Antigen preparation. Confluent monolayers of HEp-2 cells were inoculated with 10^6 50% tissue culture infective doses of wild strains of HSV-1 or HSV-2 typed by direct immunofluorescence with monoclonal antibodies (Syva Microtrak, HSV-1/HSV-2 culture confirmation/typing test; Syva Co.). Uninfected cell extracts were also prepared in an identical manner. After virus adsorption for 1 h, the infected cells were washed with phosphate-buffered saline (PBS) and fresh Eagle's minimal essential medium with 1% fetal calf serum (Commonwealth Serum Laboratories, Melbourne, Australia) was added. When a complete cytopathic effect developed (approximately 24 to 48 h), the cells were scraped into the medium and were pelleted by centrifugation at 500 × *g* for 10 min at 4°C. After two washings in PBS, the cells were disrupted in a Dounce homogenizer and were resuspended in 2× buffer (0.125 M Tris buffer [pH 6.8], 20% glycerol, 8% sodium dodecyl sulfate [SDS], 10% 2-mercaptoethanol [2ME], 0.025% bromophenol blue) before storage at −70°C. The protein content of the antigen was determined by the assay of Bradford (11) (Bio-Rad Laboratories, Richmond, Calif.) and adjusted to approximately 2.0 mg/ml.

(ii) Electrophoresis. The electrophoretic separation of proteins (SDS-polyacrylamide gel electrophoresis) was performed by the procedure of Laemmli (28). The transfer of proteins to nitrocellulose (NC) and immunodetection were mostly performed as described previously (6, 7), the main difference being the use of biotinylated anti-human immunoglobulin (IgG or IgM) and a peroxidase-streptavidin conjugate. The HSV-infected cell lysates were thawed and boiled for 5 min, and 60 μl was loaded onto a 1.5-mm discontinuous 9% polyacrylamide gel containing 0.1% SDS and cross-linked 2.6% bisacrylamide. After electrophoresis at 30 mA (model 500/200 power supply; Bio-Rad) for 4 to 5 h in the Protean II Slab Gel System (Bio-Rad), the gels were equilibrated for 30 min in transfer buffer (25 mM Tris base, 192 mM glycine, 20% methanol and 0.05% SDS) and were then transferred to NC at 4°C at 100 mA (model 200/2.0 power supply; Bio-Rad) overnight in a Trans-Blot Cell chamber (Bio-Rad). The NC was cut into 3-mm-wide strips and stored at 4°C.

(iii) Immunodetection. The NC strips were placed into each trough of the Small Incubation Tray (Bio-Rad) and were washed three times with PBS–Tween 20 (0.05% Tween 20 in PBS), and then 25 μl of serum diluted 1:100 in Blotto

(5% nonfat skim milk, 0.01% antifoam A in PBS) was added to each trough and the tray was incubated overnight at room temperature with rocking. The NC strips were washed three times with PBS–Tween 20 for 5 min each time with rocking, and a 1:1,500 dilution of biotinylated anti-human IgG or biotinylated anti-human IgM (Amersham Laboratories, Buckinghamshire, England) was added to each trough and the tray was incubated for 2 h with rocking at room temperature. Following three washes with PBS–Tween 20, a 1:1,000 dilution of streptavidin-horseradish peroxidase conjugate (Amersham Laboratories) was added to each trough and the tray was incubated for 2 h with rocking at room temperature. After three washes with PBS–Tween 20 the strips were stained with 4-chloro-1-naphthol (Sigma Chemical Co., St. Louis, Mo.) substrate (60 mg of 4-chloro-1-naphthol in 20 ml of cold methanol mixed with 60 μl of cold 30% H_2O_2 in 100 ml of TBS [Tris-buffered saline; 500 mM NaCl in 20 mM Tris; pH 7.5]) prepared just before use. After 10 to 15 min of incubation at room temperature with rocking, the strips were washed three times with distilled water and were allowed to dry.

Depletion of IgG and rheumatoid factor from serum prior to testing for specific IgM by WBA. All sera analyzed by WBA for specific IgM antibody were depleted of IgG and rheumatoid factor prior to testing by treatment with sheep anti-human IgG (gamma globulin) (rheumatoid factor absorbent; Behringwerke AG, Marburg, Germany) (19) in accordance with the manufacturer's instructions.

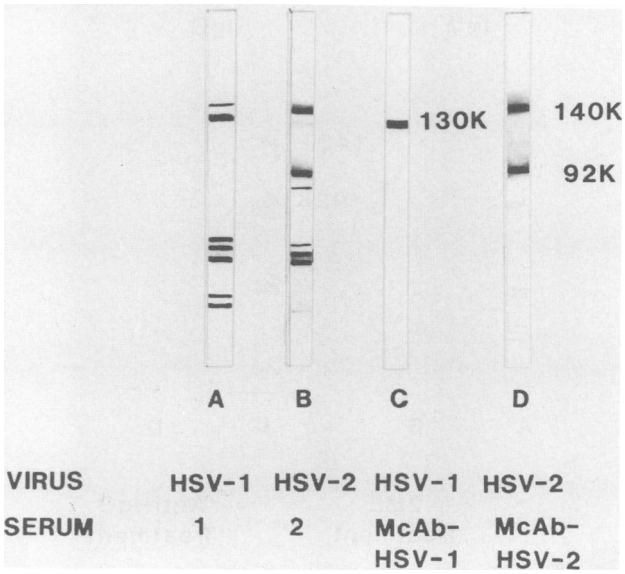

FIG. 1. Type-specific glycoproteins to HSV-1 and HSV-2 were characterized by WBA as those reacting with monoclonal antibodies (McAbs) to the HSV-1 130-kDa gpC (gC-1) (lane C) and HSV-2 92-kDa (gpG-2) and 140-kDa glycoproteins (lane D) in HSV-1- and HSV-2-infected cell lysates, respectively. Serum from patient 1 with proven genital HSV-1 reacted with the 130-kDa gpC-1 (lane A) and serum from patient 2 with proven genital HSV-2 (lane B) reacted with the 92-kDa (92K) gpG-2- and 140-kDa (140K)-specific glycoproteins.

RESULTS

Characteristics of HSV type-specific glycoproteins by WBA with specific monoclonal antibodies. The type-specific glycoproteins were characterized by WBA according to their apparent molecular sizes with murine-specific monoclonal antibodies. As expected, a 130-kDa gpC (gpC-1) was identified as HSV-1 specific, and 92-kDa gpG-2 and 140-kDa glycoproteins were identified as HSV-2 specific (Fig. 1, lanes C and D). These viral glycoproteins were not recognized by normal mouse or normal rabbit serum, and monoclonal antibody specific to HSV-1 or HSV-2 and hyperimmune rabbit antiserum to HSV-1 or HSV-2 did not recognize similar glycoproteins in blots of uninfected cell lysates (data not shown). Human antibodies to HSV-1 and HSV-2 were characterized by their reactivities with the type-specific glycoproteins in parallel with these monoclonal antibodies. Type-specific antibodies to HSV-1 were defined as those which reacted only with the HSV-1-specific 130-kDa gpC (gpC-1) (Fig. 1, lane A), and type-specific antibodies to HSV-2 were defined as those which reacted only with HSV-2-specific 92-kDa gpG-2 and 140-kDa glycoproteins (Fig. 1, lane B).

Specificity of IgM and IgG WBA. To assess the specificity of the IgM WBA, serum from a patient with proven primary genital HSV-2 infection and specific IgM and IgG antibodies to gpG-2 by ELISA (20) was treated with an equal volume of 0.5 M 2ME in PBS at 37°C for 1 h and was then tested in parallel with an untreated serum sample by the WBA for HSV-2-specific IgM. It was demonstrated that specific IgM reacted strongly with gpG-2 by WBA before treatment (Fig. 2, lane A), but not after treatment (Fig. 2, lane B). After 2ME reduction of IgM, residual antibody did not react with anti-human IgM (μ chain) biotinylated conjugate (Amersham Laboratories). Hence, the IgM conjugate was not contaminated with anti-IgG (gamma globulin) antibody. This excluded the possibility that any residual specific IgG following IgG depletion caused false-positive results in the IgM WBA.

To assess the specificity of the IgG WBA, the serum was treated with anti-human IgG (gamma globulin) to deplete the IgG and was then analyzed for specific IgG. No specific IgG reactive with either the high-molecular-size specific glycoproteins or the low-molecular-size viral polypeptides was detected (Fig. 2, lane D). This demonstrated that the IgG conjugate was gamma chain specific and was not contaminated with anti-μ chain antibody. However, untreated serum reacted strongly with the glycoproteins (Fig. 2, lane C).

None of the three serum specimen pairs from the patients with primary HSV-1 infection reacted with the HSV-2-specific gpG-2 in both the IgM and the IgG tests. However, all three serum specimen pairs showed seroconversion to the 130-kDa gpC-1 band, and both serum specimens of each pair reacted with the type-common low-molecular-size polypeptides in both the IgM and IgG tests.

Determination of specific IgM and IgG in primary HSV-2 genital infection. Paired and sequential serum specimens obtained from 17 patients with primary HSV-2 genital infections (confirmed by HSV-2 isolation) were analyzed by WBA and demonstrated both IgM and IgG seroconversion to the HSV-2-specific glycoproteins in all patients between 7 and 10 and 14 and 28 days of the onset of symptoms (Table 1). None reacted with the HSV-1-specific 130-kDa (gpC-1) glycoprotein. The time of appearance of IgM antibody directed against the gpG-2 glycoproteins and type-common low-molecular-size viral polypeptides ranging from 40 to 65 kDa is shown in Table 1. IgM against gpG-2 was not detected in any acute-phase serum specimen collected within 10 days of the onset of symptoms. However, HSV-2-specific IgM was detected in all 17 serum specimens collected from 14 to 28 days after the onset of symptoms and in 2 additional

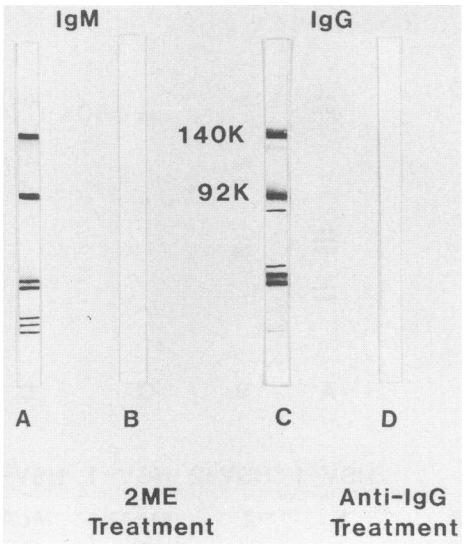

FIG. 2. Specificity of IgM and IgG WBA. To assess the specificity of the IgM WBA, serum from a patient with proven primary genital HSV-2 infection and specific IgM and IgG antibodies to gpG-2 was treated with 2ME. Specific IgM reacted strongly with the 92-kDa (92K) gpG-2 and 140-kDa (140K) glycoproteins before treatment (lane A), but not after treatment (lane B). To assess the specificity of IgG, the serum was treated with anti-human IgG (gamma globulin) to deplete IgG. Specific IgG reacted strongly with the 92-kDa gpG-2 and 140-kDa glycoproteins before treatment (lane C), but not after treatment (lane D).

serum specimens collected from two patients at 30 to 48 days after the onset of symptoms. However, of two serum specimens collected at 70 to 80 days after the onset of symptoms, neither reacted with gpG-2 but both reacted with the low-molecular-size proteins. IgM antibody to low-molecular-size viral polypeptides was demonstrated in 13 of 17 (76%) acute-phase serum specimens taken within 10 days after the onset of symptoms. Of the four acute-phase serum specimens in which IgM could not be detected, three were collected earlier (within 2 days of onset) and one was collected between 3 and 7 days of the onset of symptoms.

IgG antibody to gpG-2 was not detected in acute-phase serum specimens collected within 10 days of the onset of symptoms. However, IgG antibody to gpG-2 as well as IgG antibody to the type-common low-molecular-size 40- to 65-kDa proteins was detected in all 17 convalescent-phase serum specimens taken at 14 to 28 days of the onset of symptoms and in 2 serum specimens collected at 30 to 48 days and 70 to 80 days of the onset of symptoms. Early IgG antibodies to low-molecular-size viral polypeptides were demonstrated in 6 of 17 (35%) acute-phase serum specimens taken within 10 days of the onset of symptoms. The 11 serum specimens in which IgG to low-molecular-size viral polypeptides was not detected were all taken earlier and within 7 days of the onset of symptoms. None of the 38 acute-phase or early- or late-convalescent-phase serum specimens reacted with gpC-1.

A representative immunoblot staining pattern from one patient depicting the appearance and decline of IgM antibodies to the gpG-2 glycoproteins during the acute and convalescent phases of the illness is shown in Fig. 3. A progressive decline of IgM antibodies in the convalescent phase of the illness was observed, so that they were still present at 45 days but were not detectable at 74 days after the onset of symptoms. The convalescent phase of the disease was also heralded by the progressive rise of IgG antibodies to the virus-specific glycoproteins. However, as expected, IgG antibodies were still present at 74 days after the onset of symptoms. The kinetics of the IgM and IgG antibody responses described here were consistently observed during the acute and convalescent stages following all primary infections investigated in the present study.

Determination of specific IgM and IgG in patients with recent initial nonprimary HSV-2 genital herpes. The majority (61%) of the 23 patients with initial nonprimary HSV-2 genital infection from whom acute-phase sera only were available were found to have no HSV-2-specific IgM response but had only an IgG response to gpG-2. gpG-2 IgM only (no IgG) was detected in the remainder (39%) of the 23 patients. All of the 23 serum specimens contained HSV-1-specific IgG (but not IgM) antibodies which reacted with gpC-1 in the WBA. IgM and IgG antibodies to type-common low-molecular-size viral polypeptides of HSV-1 and HSV-2 were detected in all 23 serum samples. No follow-up serum samples were available from these patients.

Determination of specific IgM and IgG in patients with recurrent HSV-2 genital herpes. The IgM response in patients with recurrent HSV-2 infections was not type specific. Specific IgM to gpG-2 was not demonstrated in any of the 12 serum specimen pairs from patients with proven recurrent HSV-2 genital herpes. However, IgM antibodies to type-common low-molecular-size viral polypeptides of HSV-1

TABLE 1. Onset, seroconversion, persistence, and decline of IgM and IgG antibodies to specific 92- and 140-kDa and type-common low-molecular-size (40- to 65-kDa) glycoproteins in 17 patients with proven primary HSV-2 genital infections

Serum specimen and day of collection after onset of symptoms	No. of serum specimens	No. of reactive serum specimens/total no. tested			
		IgM WBA		IgG WBA	
		92- and 140-kDa glycoprotein	Low-molecular-size glycoprotein	92- and 140-kDa glycoprotein	Low-molecular-size glycoprotein
Acute phase (n = 17)					
<2	3	0/3	0/3	0/3	0/3
3–7	8	0/8	7/8	0/8	0/8
7–10	6	0/6	6/6	0/6	6/6
Convalescent phase (n = 21)					
14–28	17	17/17	17/17	17/17	17/17
30–48	2	2/2	2/2	2/2	2/2
70–80	2	0/2	2/2	2/2	2/2

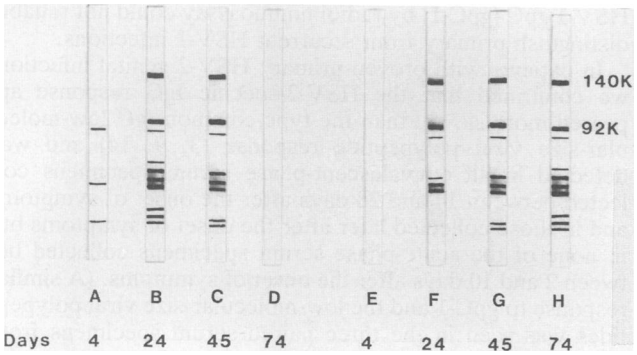

FIG. 3. Representative immunoblot staining pattern from a patient depicting the appearance and decline of IgM and IgG to 92-kDa (92K) gpG-2 and 140-kDa (140K) glycoproteins during the acute and convalescent phases of proven primary genital HSV-2 infection. In the convalescent phase, a progressive decline of IgM (lanes A, B, C, and D) and a rise of IgG (lanes E, F, G, and H) to the specific glycoproteins was shown.

and HSV-2 were demonstrated in all serum specimen pairs. Specific IgG antibodies reacted strongly to both gpC-1 and gpG-2 glycoproteins as well as type-common low-molecular-size viral polypeptides in all acute- and convalescent-phase serum specimens. The same strong reaction was also seen in the third serum specimen collected at 30 to 45 days after the onset of symptoms from two patients. The immunoblot staining patterns of IgG antibody in paired serum specimens from a patient with a representive case of recurrent HSV-2 genital infection are shown in Fig. 4. No significant visual difference in intensity of the staining of the gpG-2 glycoproteins in acute- and convalescent-phase sera was observed.

Determination of HSV-2-specific IgM and IgG in the general population and high-risk group. Twenty-five serum specimens from randomly selected children between 1 and 9 years of age (mean, 6.5 years), 45 serum specimens from healthy adult Westmead Hospital laboratory staff with no history of genital herpes, and 30 serum specimens from randomly selected patients attending the Sydney and Parramatta STD clinics (as the high-risk group) were analyzed by WBA. None of the 25 serum specimens from children reacted with the gpG-2 species, but 18 (72%) serum specimens had IgG to gpC-1, indicating that most individuals were infected with HSV-1 early in life. Of 45 serum specimens from the healthy adult hospital staff, 40 (88.9%) had IgG (but no IgM) to gpC-1, indicating that the majority of the adult population have been infected with HSV-1 in the past. Only 2 (4.4%) of 45 serum specimens from the healthy adult laboratory staff had IgG (but no IgM) to gpG-2, suggesting a low prevalence of HSV-2 in this group. In contrast, all 30 (100%) serum specimens from the high-risk group had IgG to gpC-1, and 15 (50%) of 30 serum specimens had IgG to gpG-2. IgM to gpC-1 or gpG-2 glycoproteins was not demonstrated in the high-risk group. The results indicate that half of these patients had past HSV-2 infections that were presumably acquired sexually.

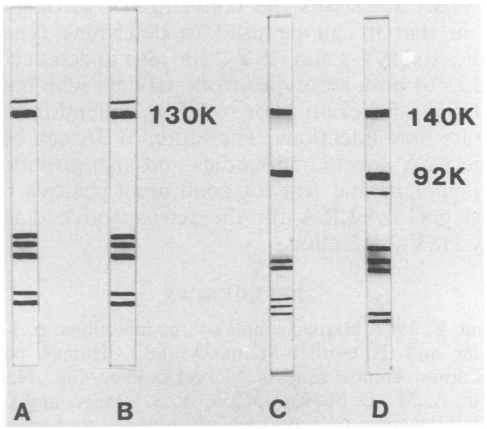

FIG. 4. Immunoblot staining pattern of HSV-2 IgG antibody (lanes C and D) in paired serum specimens from a representative patient with recurrent HSV-2 genital infection with preexisting HSV-1 antibody (lanes A and B). There was no significant difference in the staining intensity of the glycoproteins between the sera. Acute-phase (lanes A and C) and convalescent-phase (lanes B and D) sera were tested. 130K, 140K, and 92K, 130-, 140-, and 92-kDa glycoproteins, respectively.

DISCUSSION

This appears to be the first report that IgM antibody directed against the HSV-2 type-specific gpG-2 glycoprotein detected by WBA can be used for the diagnosis of recent primary HSV-2 genital infections. WBA also appears to have a place in the diagnosis of recent nonprimary initial genital herpes infection, but it cannot be used for the retrospective diagnosis of recent recurrent infections.

Furthermore, the HSV-2 specificities of the 92-kDa and 140-kDa forms of gpG-2 were confirmed, and further evidence was provided for the HSV-1 specificity of the 130-kDa gpC-1 glycoprotein. The HSV-2-specific monoclonal antibody reacted with two sets of glycoproteins with molecular sizes of 92 and 140 kDa in the WBA (Fig. 1), and this may represent components of gpG-2 at different stages of maturation (e.g., precursor or product). However, a monoclonal antibody that reacts specifically with an HSV-2-specific induced protein with a molecular size of 140 kDa has been reported (34). Not surprisingly, it has been reported that the HSV-1 counterpart (gpG-1) also is detected as two sets with molecular sizes of 40 to 44 and 60 to 88 kDa against specific monoclonal antibody to HSV-1 (31).

The 92-kDa gpG-2 confirmed as HSV-2 specific in the present study has also been reported by others (32, 35, 40). We found that paired serum specimens from patients with confirmed cases of primary HSV-1 infection did not cross-react with the 92-kDa gpG-2 and 140-kDa bands. This specificity is further shown by the results of testing of sera from 25 children, none of whom had detectable HSV-2 antibody but 72% of whom had IgG to HSV-1 (gpC-1). Similarly the low prevalence of HSV-2 (gpG-2) antibody (4.4%) in healthy laboratory staff was in contrast to the 50% prevalence of HSV-2 antibody in the STD clinic attendees. These results are similar to those obtained by the gG-2 immunodot enzymatic assay of Lee et al. (30) and our own conventional indirect ELISA for gpG-2 (20).

gpC-1 (45, 46) and gpC-2 (37, 46) are colinear genes encoding viral glycoproteins and have both cross-reactive and type-specific antigenic epitopes. Recognition of the relative type specificity of human gpC-1 antibodies is facilitated because gpC-1 (130 kDa) and gpC-2 (75 kDa) have different molecular sizes, so they can easily be distinguished in Western blots of HSV-1 and HSV-2. Nevertheless, the limited cross-reactivity of anti-gpC-2 for some of the epitopes in gpC-1 might be expected and has been reported previously (31, 41, 42, 47).

Results of our studies agree with those of other investigators (2, 13, 17, 45), in that gpC-1 is usually specific because none of the 17 serum specimen pairs from patients with primary HSV-2 genital infections demonstrated reactivity in the WBA with gpC-1. Although this is a small sample, these results suggest that the common amino acid sequences in gpC-1 and gpC-2 code for relatively weak or infrequently recognized antigens and that the immunodominant antigens in these amino acids are type specific under the rather harsh denaturing conditions of WBA (48).

It is apparent that the main use of HSV-2-specific IgM testing is in the diagnosis of primary genital herpes. Although HSV-2 IgG as well as IgM seroconversion was demonstrated in all 17 patients with primary infection, the advantage of IgM is that it enables a retrospective type-specific diagnosis to be made on a single convalescent-phase serum specimen collected between 14 and 28 days after the onset of disease.

The majority of patients (61%) with initial nonprimary HSV-2 genital infection were found to have an HSV-2-specific IgG response but no HSV-2-specific IgM response. This indicated that most of these patients either could have had an earlier unnoticed HSV-2 clinical episode or, alternatively, they may have previously been asymptomatically infected with HSV-2. Although most HSV-2 antibody-positive people are unaware of their infection (21), half of such women admit to having had previously unrecognized symptomatic lesions (29). Only a minority of patients (39%) with initial nonprimary HSV-2 genital infection produced an HSV-2 IgM response (but no IgG response). This suggests that these patients were not previously infected with HSV-2. For this reason, the interpretation of IgM test results requires a careful correlation with patient history and the patient's clinical course. Later sera were unavailable for testing for IgG seroconversion. IgG antibodies to HSV-1 gpC (gpC-1) were present in all patients in this group, and although anti-HSV-2 antibody did not react with gpC-1 in the group with proven primary HSV-2 infection, we cannot exclude the cross-reactivity of anti-HSV-2 antibody for gpC-1 in the occasional patient.

Our study showed that the IgM response in patients with recurrent HSV-2 infection is not type (gpG-2) specific. However, we found IgM to only type-common low-molecular-size polypeptides of HSV-1 and HSV-2 both in patients with recurrent infections and also in most patients with initial nonprimary infections, which were probably also recurrent. Therefore, the use of commercial indirect immunofluorescence assay or other standard IgM procedures, which are incapable of differentiating between cross-reacting and specific IgM antibodies, is not specific or relevant for the diagnosis of initial nonprimary or recurrent HSV-2 infection (15). The presence of IgM and IgG antibodies to type-common low-molecular-size viral polypeptides in patients with nonprimary first episode and recurrent HSV-2 infections may be due to an anamnestic response against type-common HSV antigens (9, 33, 36).

It has been reported that total HSV-specific IgM as detected by immunoassays (which use a mixture of both cross-reacting and specific antigens) is not detected in patients with recurrent infections (25) except in those which are clinically severe (22, 24, 27, 43). Total HSV IgM antibodies were a poor predictor of the acute phase of disease and long-term outcome in newborns with documented HSV infection (23). In contrast to our results, Arvin et al. (2) have reported that the detection of type-specific IgM antibody to HSV-1 gpC (gpC-1) by radioimmunoassay could not reliably distinguish primary from recurrent HSV-1 infections.

In patients with proven primary HSV-2 genital infection, we confirmed that the HSV-2-specific IgG response appeared more slowly than the type-common IgG low-molecular-size viral polypeptide response (3, 9, 14) and was detected in all convalescent-phase serum specimens collected between 14 and 28 days after the onset of symptoms and in those collected later after the onset of symptoms but in none of the acute-phase serum specimens collected between 2 and 10 days after the onset of symptoms. (A similar response to gpC-1 and the low-molecular-size viral polypeptides was seen in the three paired serum specimens from patients with primary HSV-1 infection.) In patients with recurrent HSV-2 infections, the IgG reactivity to all HSV proteins between acute- and convalescent-phase sera was unchanged. Similar results were obtained by others (7, 9). The present study confirms that the low-molecular-size polypeptides are type common.

It has been reported that preexisting HSV-1 antibody may result in the lack of clarity of specific gpG-2 bands in WBA (because of the anamnestic response to type-common antigens) (33, 36), requiring repeat serologic testing after adsorption with HSV-1 (8). A similar disproportionate HSV-1 staining may occur in patients with recurrent HSV-2 episodes (7, 9, 10). In our study, the 92-kDa gpG-2 band was used as the sole marker of HSV-2 infection because of potential difficulty in distinguishing antibody which reacts against the 130-kDa gpC-1 from that which reacts with the 140-kDa form of gpG-2. Therefore, cross-adsorption was not necessary. The 140-kDa band is an additional HSV-2-specific marker which, in our experience, is almost always found together with the 92-kDa gpG-2. The specific staining patterns were easily and reliably determined visually, and quantitation by densitometric scanning was unnecessary. This may have been because of our use of a 1:100 dilution of serum combined with the use of the biotin-avidin-amplified antibody detection system. Apart from adding time and additional cost to the assay (7), cross-adsorption of sera with HSV-1 antigens may also deplete heterologous antibody titers and complete adsorption of cross-reacting antibody is often very difficult (30).

The WBA surpasses the conventional serological techniques in that it can be used to determine type-specific antibodies to HSV-1 and HSV-2 and also to detect coexisting antibodies to both serotypes from patients who had experienced HSV-1 infection prior to HSV-2 infection (1, 13, 38, 44) or rare dual infections. Therefore, WBA can be used to serotype HSV-specific antibodies and also provides an excellent supplemental test for confirming positive results in the IgM gpG-2 ELISA for the retrospective diagnosis of primary HSV-2 infection.

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