JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1991, p. 778–781
0095-1137/91/040778-04$02.00/0

Vol. 29, No. 4

Seroreactive Recombinant Herpes Simplex Virus Type 2-Specific Glycoprotein G

DEBORAH L. PARKES, CARISSA M. SMITH, JOANNE M. ROSE,† JOHN BRANDIS, AND STEPHEN R. COATES†*

Triton Biosciences Inc., Alameda, California 94501

Received 26 March 1990/Accepted 8 January 1991

The herpes simplex virus type 2 (HSV-2) genome codes for an envelope protein, glycoprotein G (gG), which contains predominantly type 2-specific epitopes. A portion of this gG gene has been expressed as a fusion protein in *Escherichia coli*. Expression was regulated by a lambda phage p_L promoter. The 60,000-molecular-weight recombinant protein was purified by ion-exchange chromatography. Amino acid sequence analysis confirmed the N terminus of the purified protein. Mice immunized with recombinant gG developed antibodies reactive with native HSV-2 protein, but not with HSV-1 protein, in an indirect immunofluorescence assay. The serological activity of this purified recombinant gG protein was evaluated by immunoblot assay. This protein was reactive with an HSV-2 gG monoclonal antibody. It was also reactive with HSV-2 rabbit antiserum but not with HSV-1 rabbit antiserum. Of 15 patient serum samples known to have antibody to HSV-2, 14 were reactive with this recombinant type 2-specific gG protein, and none of 15 HSV antibody-negative patient serum samples showed reactivity. In agreement with the expected prevalence of HSV-2 infection, 27.6% of 134 serum samples from random normal individuals had antibodies reactive with recombinant gG. This recombinant gG protein may be of value in detecting HSV-2-specific antibody responses in patients infected with HSV-2.

Herpes simplex virus type 1 (HSV-1) and HSV-2 are highly related viruses. Many of their counterpart gene products have similar molecular weights and common antigenic determinants (8, 10, 12). Yet clinical manifestations of HSV-1 and HSV-2 differ. The majority of primary genital herpes infections and recurrent genital herpes infections are caused by HSV-2. Nongenital herpes infections such as common cold sores are caused primarily by HSV-1 (3).

Because of the strong serological cross-reactivity between HSV-1 and HSV-2, serological diagnosis of infection with HSV-2, especially in persons who have been infected with HSV-1 as well, has been technically very difficult (4, 8, 12).

Recently, HSV-2 glycoprotein G (gG) (9, 13) has been used as an antigen to determine type 2-specific antibody responses. A monoclonal antibody to native HSV-2 gG was used to affinity purify gG from viral lysate antigen preparations for use in the type 2-specific assay (7). This assay has been useful in epidemiological studies (5), in the diagnosis of HSV-2 infections, and in the screening of pregnant women (1, 7, 15) and potential semen donors (11).

HSV-1 gG has been shown to be distinctly different from HSV-2 gG (7, 9, 13). However, HSV-2 gG and HSV-1 gG have similar signal sequences at their amino termini. In addition, at their carboxyl termini they have segments of 153 homologous amino acids which contain the putative transmembrane anchor domains. HSV-2 gG, however, contains an additional unique segment of 526 amino acids. This unique segment contains the putative type 2-specific epitopes observed with this glycoprotein (10).

We report here the cloning and expression in *Escherichia coli* of this unique type 2-specific region of HSV-2 gG. In addition, we describe the purification and serological activity of this HSV-2-specific recombinant protein.

* Corresponding author.
† Present address: Chiron Corp., 4560 Horton St., Emeryville, CA 94608-2916.

MATERIALS AND METHODS

Plasmids. A plasmid consisting of the *Hin*dIII I fragment of HSV-2 HG52 inserted into the *Hin*dIII site of pAT153 was obtained from D. J. McGeoch, Institute of Virology, University of Glasgow (10).

Cloning and expression. The plasmid described above was digested with restriction nucleases to isolate a fragment representing approximately 95% of the HSV-2 unique region of gG (Fig. 1). This fragment was inserted into a lambda p_L promoter-based (14) expression vector, pTBI102. The resulting plasmid contained the HSV-2 unique sequence of gG fused at its N terminus to the *E. coli trpE* leader and at its C terminus to the α-peptide sequence of β-galactosidase. The expression plasmid was transformed into an *E. coli* strain containing the lambda *c*I857 repressor. Expression of the fusion protein was induced by heat shock at 42°C for 15 min, followed by growth of the culture at 37°C.

Patient sera. Sera from patients with herpes infections confirmed by virus isolation were obtained from L. M. Frenkel, University of California at Los Angeles School of Medicine; A. Arvin and C. Prober, Stanford University; C. Gleaves, Fred Hutchinson Institute, Seattle, Wash.; B. Forgani, California State Department of Public Health, Berkeley; and J. Kettering, Loma Linda University School of Medicine, Loma Linda, Calif. The HSV type of each viral isolate was confirmed by immunofluorescent staining with type-specific monoclonal antibodies supplied by Syva Company. At the time that the serum samples were drawn, the clinical histories of the HSV-2-infected patients indicated that these infections were of the nonprimary recurrent type. Sera from patients assumed to be infected with only HSV-1 were from adults from whom HSV-1 was isolated. When the serum samples were drawn, these patients had histories of recurrent oral herpes and had no histories of genital herpes. Serum specimens from normal healthy adults were obtained from volunteer blood donors in Oklahoma City, Okla.

Monoclonal antibody. A monoclonal antibody to HSV-2

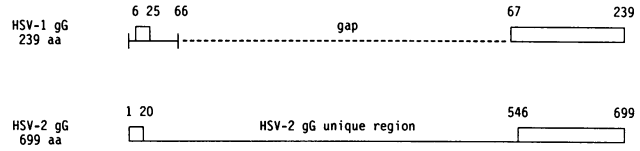

FIG. 1. Comparison of the amino acid sequences of HSV-1 gG and HSV-2 gG. The proteins are drawn to scale. The boxed areas show homologous sequences of HSV-1 gG and HSV-2 gG. HSV-2 gG has a segment of 526 amino acids not found in HSV-1 gG, as indicated. The diagrams are adapted from reference 10. aa, Amino acids.

gG was obtained from N. Balachandran, University of Kansas (2).

HSV-1 and HSV-2 antibody enzyme immunoassays. HSV-1 and HSV-2 purified antigen enzyme immunoassays were obtained from Whittaker Bioproducts and were performed according to the manufacturer's instructions.

Indirect immunofluorescence assays. HSV-1- and HSV-2-infected HEp-2 cell monolayers on microscope slides were obtained from Zeus Scientific, Inc., Raritan, N.J. BALB/c mice immunized with 5 μg of purified recombinant gG were bled 3 weeks after immunization. Preimmunization serum samples and immunized serum samples were diluted and assayed by indirect immunofluorescence according to the manufacturer's instructions.

Purification. The *E. coli* cell pellet was resuspended in 50 mM Tris, pH 8.1, and lysozyme was added to 0.2 mg/ml. The suspension was kept on ice for 1 h and was then frozen at −70°C for 2 h. After thawing, the lysate was adjusted to 2 mM $MgCl_2$, and DNase I was added to 5 μg/ml. The lysate was kept on ice for 30 min and was then centrifuged at 17,000 × *g* for 30 min at 4°C. The pellet (pellet 1) was resuspended by sonication in 50 mM Tris (pH 8.1)–5 mM EDTA–5 mM ethyleneglycol-bis(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) containing nonionic detergents. This material was centrifuged, and the resulting pellet was solubilized in a chaotropic agent. The recombinant protein was subsequently purified by ion-exchange chromatography.

Amino acid sequence analysis. A 100-μl sample containing approximately 10 μg of recombinant gG was mixed with an equal volume of dialysis buffer (25 mM ammonium bicarbonate, 0.01% sodium dodecyl sulfate [SDS]) and dialyzed against 2 liters of dialysis buffer at room temperature in a 10,000-molecular-weight-cutoff Collodion bag (Schleicher and Schuell). The sample was then centrifuged under a vacuum until dry. The dried sample was reconstituted in 65 μl of 0.1% trifluoroacetic acid. Five microliters was analyzed by gel electrophoresis, and the remaining material was loaded (two aliquots of 30 μl each) onto an Applied Biosystems sequencing filter which had been precycled with 1.5 mg of Biobrene (Applied Biosystems). Amino-terminal sequence analysis was performed through 25 cycles on an Applied Biosystems model 475 gas phase sequencer with on-line detection of phenylthiohydantoin amino acids.

Immunoblot procedure. Purified recombinant gG was electrophoresed on SDS–8% polyacrylamide slab gels. Proteins were electrophoretically transferred onto nitrocellulose for 16 h at 60 mA by using transfer buffer composed of 25 mM Tris hydrochloride and 192 mM glycine. The nitrocellulose was blocked for 20 min in 1 M glycine–5% (wt/vol) nonfat dry milk–1% (wt/vol) ovalbumin (blocking buffer) and was then incubated overnight at 4°C with serum diluted 1:100 in blocking buffer. After three 3-min rinses in phosphate-buffered saline containing 0.05% Tween 20, the nitrocellulose was incubated for 2 h at room temperature with horseradish peroxidase-labeled goat anti-human immunoglobulin G and 10% fetal bovine serum in phosphate-buffered saline containing 0.05% Tween 20. The nitrocellulose was again rinsed and was then developed in substrate-chromogen solution containing 0.2 mg of 3,3′-diamino-benzidine-4HCl per ml, 0.02% (wt/vol) $NiCl_2$, and 0.05% (wt/vol) H_2O_2 in 10 mM Tris hydrochloride, pH 7.5. The reaction was stopped by rinsing the nitrocellulose in water.

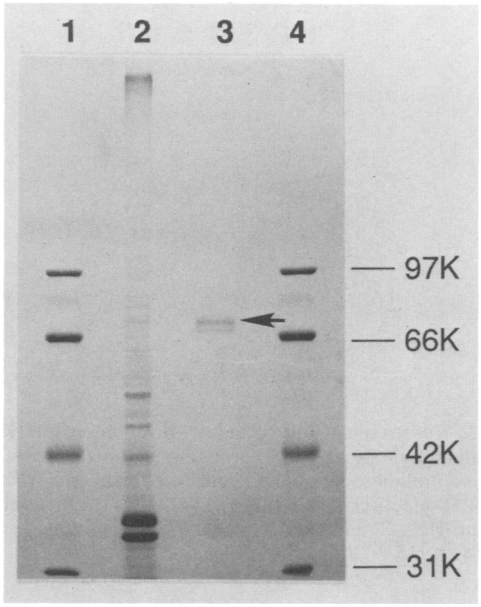

FIG. 2. Purification of recombinant HSV-2-specific gG. Samples were applied to an SDS–8% polyacrylamide gel and stained with Coomassie blue. Lanes: 1 and 4, molecular weight markers as indicated; 2, detergent-extracted insoluble pellet from *E. coli*; 3, purified recombinant HSV-2-specific gG (arrow). Molecular weights are given in thousands.

RESULTS

Purification. Purity of greater than 80%, as estimated by visual inspection of Coomassie blue-stained SDS-polyacrylamide gels, was achieved for recombinant HSV-2-specific gG (Fig. 2). The recombinant protein, which appeared as a doublet, had an estimated molecular weight of 60,000 when electrophoresed to equilibrium on gradient SDS-polyacrylamide gels (3 to 18% polyacrylamide) (data not shown).

Sequence analysis. Sequence analysis of the purified protein confirmed the presence of the *trpE* leader sequence of the recombinant fusion protein.

Recombinant-gG-immunized mice. Mice immunized with purified recombinant gG produced antibodies that reacted with HSV-2-infected cell monolayers in an indirect immunofluorescence assay. The cytoplasmic immunostaining pattern observed was identical to that produced by a native HSV-2 gG monoclonal antibody (data not shown). No reactivity was observed with HSV-1-infected monolayers. Preimmune mouse serum was not reactive with either HSV-1- or HSV-2-infected cell monolayers.

Serological evaluation by immunoblot assay. A monoclonal antibody produced to HSV-2 gG was reactive with the recombinant protein by immunoblot assay. Rabbit antiserum

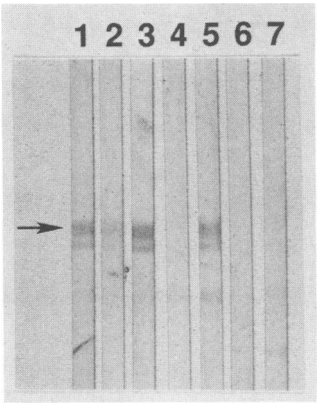

FIG. 3. Reactivity of antibodies with recombinant HSV-2-specific gG in immunoblot assay. Purified antigen was probed with the following antibodies: lanes 1, 2, and 3, human sera from patients with HSV-2 infections; lane 4, rabbit anti-HSV-1 antiserum; lane 5, rabbit anti-HSV-2 antiserum; lanes 6 and 7, human sera from patients with HSV-1 infections only.

produced against whole HSV-1 was not reactive with the recombinant protein; however, rabbit antiserum produced against HSV-2 was reactive (Fig. 3). In agreement with results of Coomassie blue staining, the immunoblot reactivity appeared as a doublet. As shown in Table 1, 95% of patients with prior HSV-2 infection had antibodies reactive to recombinant gG. No antibodies to recombinant gG were detected in patients with prior HSV-1 infection or in patients without prior HSV-1 or HSV-2 infection. Antibodies to the recombinant protein were detected in only 27.6% of 134 serum specimens from random normal individuals. In contrast, both the HSV-1 and HSV-2 antigen enzyme immunoassays detected antibodies in 66.4% of these serum specimens. A total of 33 of the 134 serum specimens were negative by both the HSV-1 and HSV-2 antigen enzyme immunoassays. No antibody to recombinant gG was detected in these seronegative samples.

DISCUSSION

These studies represent the first reported expression of the type 2 unique sequence of HSV-2 gG in procaryotic cells. The successful expression of HSV-2-specific epitopes was demonstrated by the reactivity of the recombinant gG with a monoclonal antibody to HSV-2 gG, with anti-HSV-2 rabbit polyclonal antibodies, and with antibodies from HSV-2-infected patients. In addition, mice immunized with purified recombinant protein produced antibodies reactive with HSV-2 native protein. On the basis of its sequence, this recombinant gG should contain more than 90% of any type 2-specific linear epitopes found on HSV-2 gG. When used in the immunoblot assay, it is completely denatured; all of these linear epitopes should be exposed. However, under these conditions, the recombinant gG may have lost some conformational epitopes. This may decrease its sensitivity relative to that of native gG.

TABLE 1. Serological reactivity of recombinant HSV-2-specific gG with patient sera

Patient group	No. of patients	% Positive[a]
Prior HSV-2 infection[b]	15	95
Prior HSV-1 infection only[c]	9	0
No prior HSV-1 or HSV-2 infection[d]	15	0

[a] Reactivity was determined by immunoblot assay with recombinant HSV-2-specific gG.
[b] Prior HSV-2 infection was established by virus isolation.
[c] Prior infection only with HSV-1 was established by isolation of virus from patients with oral herpes and by no clinical history of genital herpes.
[d] The absence of prior HSV-1 or HSV-2 infection was established by the absence of HSV-1 or HSV-2 antibodies.

An advantage of this recombinant gG over viral gG is its lack of any epitopes common to HSV-1 and HSV-2. On the basis of its sequence, HSV-2 gG may have epitopes common to HSV-1 gG on its N terminus and C terminus. McGeoch et al. (10) confirmed the presence of one epitope common to both types by using a rabbit antiserum prepared against a synthetic peptide derived from an HSV-2 gG sequence near the C terminus. This antiserum immunoprecipitated both HSV-1 gG and HSV-2 gG. The successful elimination of HSV common epitopes from this recombinant gG is shown by the failure of the gG to react with anti-HSV-1 rabbit polyclonal antibodies or with HSV-1 patient sera, as well as its lack of ability to induce HSV-1-reactive antibodies in immunized mice.

An additional advantage of recombinant gG is the economics of its production compared with that of cell culture-produced viral gG. This should facilitate its incorporation into a readily available diagnostic test.

The major value of a serological test using this recombinant is its ability to distinguish type 2-specific antibody responses in patients previously infected with HSV-1. In tests with sera from normal individuals, the recombinant gG was able to distinguish HSV-2-infected individuals from HSV-1-infected individuals. It detected antibody in 27.6% of these sera. This result agrees with the expected rate of HSV-2 infection in a normal population (5). In contrast, when these sera were tested with the HSV-1 and HSV-2 antigen enzyme immunoassays, antibody responses to HSV-1 as well as HSV-2 antigen were detected in more than 60% of these normal sera. This is the expected infection rate for HSV-1 alone (12) and is well above the expected rate of infection for HSV-2 (5). As expected, the HSV-2 antigen enzyme immunoassay detected antibody responses to antigens shared by both HSV-1 and HSV-2 and was not able to distinguish HSV-2-infected individuals.

Perhaps the most important application of recombinant HSV-2-specific gG will be to identify pregnant women at risk of transmitting HSV-2 to their infants at delivery. Perinatal transmission is infrequent but results in high morbidity and is often fatal in newborns (3, 5). Most infants with neonatal HSV-2 infection are born to mothers with no history or clinical signs of genital herpes (15). A sensitive and specific serological test for HSV-2 infection may assist in identifying pregnancies possibly complicated by recurrent maternal HSV-2 infections.

It has recently been reported that one-half of women with HSV-2-specific antibodies who reported no history of genital herpes did in fact have clinically symptomatic genital herpes (6). It has been suggested that screening certain populations of women for HSV-2-specific antibodies and subsequently instructing antibody-positive individuals on how to recognize previously unrecognized symptomatic lesions may help reduce the transmission of genital herpes.

The recent report of the transmission of HSV-2 by an asymptomatic semen donor has prompted the need for screening potential semen donors to identify those capable of transmitting HSV-2 to recipients (11). The recombinant

protein described in this report may provide an economical method for a serological donor-screening test. The complete utility of this recombinant protein in the diagnosis and screening of HSV-2 infections will be determined by further clinical investigations.

In summary, the unique type 2-specific region of the HSV-2 gG gene has been expressed in and purified from *E. coli*. This recombinant protein has been demonstrated to be sensitive in detecting HSV-2 antibodies and does not cross-react with HSV-1 antibodies. Use of this recombinant gG may provide an economical means of producing an HSV-2-specific antibody assay to diagnose and to screen for HSV-2 infections.

ACKNOWLEDGMENTS

We express our appreciation to Mark Sliwkowski, Roger Walker, Robert Akita, Mike Ferrer, and Pia Johnson for their suggestions in developing the process of purification of this recombinant protein and to Rick Harkins for performing the amino acid sequence analysis. We are also grateful to Stuart Rosenfeld, Dennis DiTullio, and Millard Cull for the large-scale fermentation required for this study.

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