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Expression of Herpes Simplex Virus Type 1 Glycoprotein I in Baculovirus: Preliminary Biochemical Characterization and Protection Studies

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We have constructed a recombinant baculovirus expressing the herpes simplex virus type 1 (HSV-1) glycoprotein I (gI). Sf9 cells infected with this recombinant virus synthesized gI-related polypeptides with apparent molecular sizes of 52 and 56 kDa. The recombinant gI appeared to be glycosylated, since it was susceptible to both tunicamycin and endoglycosidase H, and the expressed gI was transported to the surface of infected cells as judged by indirect immunofluorescence. Antibodies to the recombinant gI raised in mice neutralized HSV-1 infectivity. Finally, we show here for the first time that vaccination with gI can protect mice against HSV-1 challenge.

Herpes simplex virus type 1 (HSV-1) glycoproteins are the primary inducers and targets of humoral and cell-mediated immune responses to HSV-1 infection (12, 16). The HSV-1 glycoproteins E and I (gE and gI) form a complex that can bind the Fc portion of immunoglobulin G (1, 5, 6, 13).

To investigate the ability of immunization with gI to induce immunity to HSV-1 infection, we have expressed gI by using a baculovirus expression system. The recombinant gI was produced at high levels. It was glycosylated and was transported to the cell surface. Antibodies to the recombinant gI raised in mice neutralized the infectivity of HSV-1 in the presence of complement. Furthermore, immunization with gI protected mice against lethal intraperitoneal challenge with HSV-1. Thus, our findings suggest that the baculovirus expression system may provide a useful alternative approach to mammalian expression systems for the production of gI and that baculovirus-expressed gI might be a useful component of an HSV-1 subunit vaccine. In addition, the high level of gI expression that we achieved in baculovirus (several hundred-fold higher than in HSV-1 tissue culture infections) should allow the purification of large quantities of gI for subsequent biochemical and immunological studies.

The baculovirus transfer vector containing the complete gI open reading frame was constructed as shown in Fig. 1. Briefly, DNA representing the complete coding region of the gI gene (Fig. 1, black regions) of HSV-1 strain KOS was isolated by *Fsp*I-*Sph*I restriction enzyme digestion of plasmid pSS17 (14) and blunt-ended into the *Sma*I site of pGem-3. The plasmid pGem-gI was linearized with *Hin*dIII and digested briefly with *Bal* 31 exonuclease (to trim the 5′ end of the gI gene), and *Bam*HI linker was added. The resulting religated plasmid DNA was then cut with *Bam*HI, and the gI gene was isolated and inserted into the unique *Bam*HI site of the baculovirus transfer vector pAcYM1 (10) (Fig. 1). As was confirmed by restriction enzyme analysis and partial sequencing, this construct (pAc-gI1) contains the entire sequence of the gI gene inserted just downstream from the baculovirus polyhedrin promoter. The first ATG of the gI gene is 5 nucleotides from the polyhedrin promoter and is followed by the complete gI coding region (1,170 nucleotides) and 48 HSV-1 noncoding nucleotides after the gI termination codon.

To transfer the gI gene into the baculovirus genome, Sf9 cells were cotransfected with purified infectious baculovirus (*Autographa californica* nuclear polyhedrosis virus [AcNPV]) DNA and the pAc-gI1 transfer vector as described previously (18). Following three cycles of polyhedrin-negative plaque purification, two recombinant viruses were obtained. Both recombinants expressed gI with similar properties as determined by Western blotting (immunoblotting) using Fd69 (9), a gI-specific monoclonal antibody (a gift from S. Chatterjee). One recombinant was arbitrarily chosen for subsequent study and designated vAc-gI1. The presence of gI DNA in the baculovirus recombinant was verified by Southern blotting (data not shown).

To analyze the size of the baculovirus-expressed gI, confluent monolayers of Sf9 cells were infected with the baculovirus recombinant at a multiplicity of infection of 10 PFU per cell. Total protein extracts were run on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10% polyacrylamide) and analyzed by Western blotting as described previously (4). Two bands of 52 and 56 kDa reacted strongly with Fd69 (Fig. 2, lanes 2 and 3). These bands were more prominent at 72 h postinfection (lane 3) than at 48 h postinfection (lane 2). None of these bands was seen in wild-type-baculovirus-infected Sf9 cells (lane 1) or in uninfected Sf9 cells (data not shown).

The gI gene codes for a protein of 390 amino acids with a molecular size of 41,366 Da (11). In HSV-1-infected cells, the 41-kDa gI polypeptide is partially glycosylated to yield a precursor gI with an apparent molecular size of 55 kDa. This

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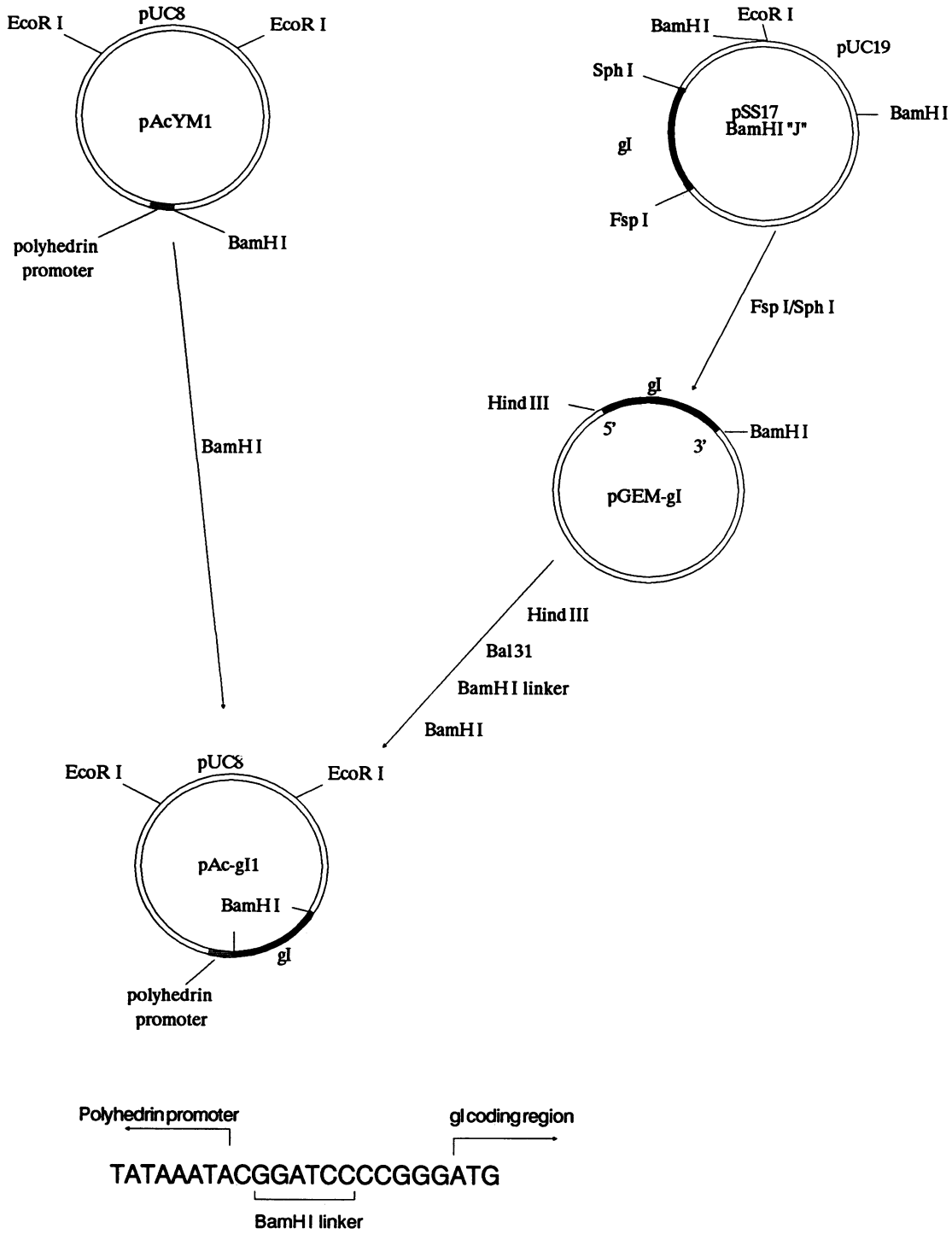

FIG. 1. Construction of the pAc-gI1 recombinant baculovirus transfer vector containing the HSV-1 gI gene. The strategy for the construction of the baculovirus transfer vector (pAc-gI1) containing the complete coding region of HSV-1 gI (■) under control of the baculovirus polyhedrin gene promoter (▩) is outlined here and described in the text. At the bottom of the figure is the sequence of the region near the 5′ end of the cloned gI in the baculovirus transfer vector. The 3′ end of the baculovirus polyhedrin gene promoter (10), a *Bam*HI linker, and the coding sequence of gI (preceded by five noncoding nucleotides of the gI gene) are indicated.

precursor gI is then further glycosylated to produce mature gI with an apparent molecular size of 65 kDa (9). Our expressed gI migrated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis with molecular sizes of 52 and 56 kDa, compared with 41 kDa for unglycosylated gI. Although this size range is consistent with a 55-kDa protein synthesized in vitro from selected HSV-1 RNA (8) and a 52-kDa protein translated from RNA transcribed by an SP6 pro-

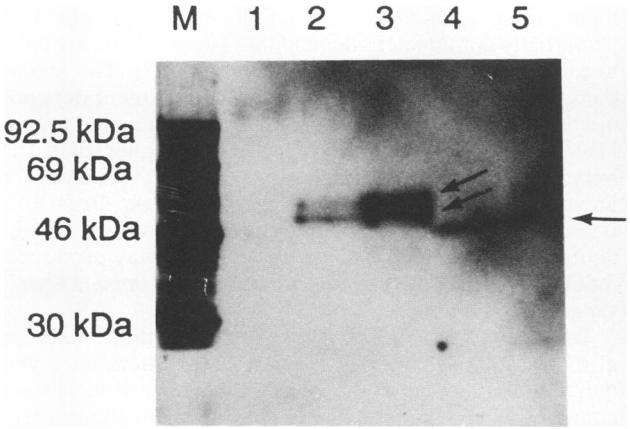

FIG. 2. Western blot analysis of expressed gI in infected insect cells. Insect cells were infected at a multiplicity of infection of 10 with the baculovirus gI recombinant (vAc-gI1), and some monolayers were treated with tunicamycin (0 to 48 h postinfection) or cell extracts were treated with endoglycosidase H as recommended by the manufacturer (Boehringer Mannheim Biochemicals). Briefly, 10^5 cells were lysed in gel sample buffer (7), sodium acetate buffer (pH 5.0) and endoglycosidase H were added, and the samples were incubated overnight. Lanes: M, molecular size markers; 1, wild-type-baculovirus-infected insect cells; 2, baculovirus-gI-infected cells 48 h postinfection; 3, baculovirus-gI-infected cells 72 h postinfection; 4, tunicamycin-treated, baculovirus-gI-infected cells (48 h); 5, endoglycosidase H-treated, baculovirus-gI-infected cells 72 h postinfection.

moter (15), gI from HSV-1-infected cells (5, 9) and from a recombinant vaccinia virus expressing gI (17) appeared to be slightly larger.

To determine whether the expressed gI underwent glycosylation, cells were subjected to tunicamycin treatment to prevent N glycosylation in infected Sf9 cells. Infected cells were treated with 4 μg of tunicamycin per ml of medium from 0 to 48 h postinfection, and total cell extracts were analyzed by Western blotting using anti-gI monoclonal antibody. The tunicamycin treatment (Fig. 2, lane 4) increased the mobility of gI relative to that of the control (lanes 2 and 3), indicating that the 52- and 56-kDa polypeptides both contain N-linked sugars. This result indicates that, like native gI (5), the untreated recombinant gI was glycosylated.

With a result similar to that of tunicamycin treatment, following endoglycosidase H treatment the 52- and 56-kDa bands were replaced by a band with an apparent molecular size of 50 kDa (Fig. 2, lane 5). Since endoglycosidase H digests high-mannose but not complex oligosaccharides, this result suggests that our baculovirus-expressed gI was N glycosylated and contained high-mannose N-glycans (non-complex saccharides).

Indirect immunofluorescence was used to determine whether the expressed gI was transported to the cell surface as was expected. Sf9 cells were infected with wild-type

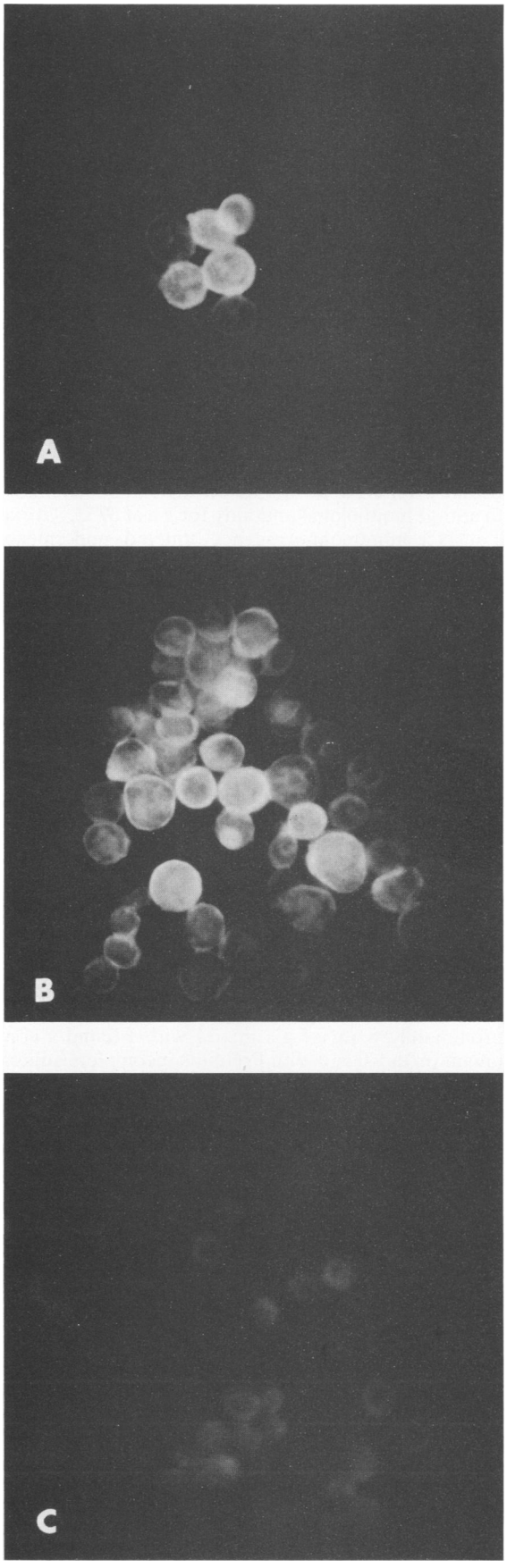

FIG. 3. Immunofluorescence of recombinant baculovirus-infected cells. Acetone-fixed or unfixed infected Sf9 cells were incubated with anti-gI monoclonal antibody followed by fluorescein-conjugated goat anti-mouse immunoglobulin G antibody and examined by fluorescence microscopy as described previously (3, 4). (A) Recombinant baculovirus-gI-infected cells, total (intracellular) fluorescence; (B) baculovirus-gI-infected cells, surface fluorescence; (C) wild-type-baculovirus-infected cells, total fluorescence.

TABLE 1. Immunization of mice with a recombinant baculovirus expressing HSV-1 gI

Immunization	No. of survivors/ total no.[a]	% Survival	Neutralization titer[b]	
			+ Complement	− Complement
Baculovirus gI	18/20	90	167	91
KOS	11/11	100	>320	>320
Mock	7/18	39	<10	<10

[a] Survival rates (protection) of the baculovirus gI recombinant- and KOS-vaccinated mice were significantly different from the mock vaccination survival rate (Fisher's exact test; $P = 0.01$).

[b] Neutralization titers are expressed as the reciprocal geometric means of the dilution that produced a 50% reduction in plaque numbers.

baculovirus (AcNPV) or recombinant baculovirus expressing gI (multiplicity of infection, 10 PFU per cell) and incubated for 72 h. As we previously described (3, 4), in order to examine total fluorescence cells were washed with phosphate-buffered saline, fixed with acetone, and incubated with anti-gI monoclonal antibody for 1 h at 37°C. To examine cell surface immunofluorescence, unfixed, unpermeabilized cells were incubated with anti-gI monoclonal antibody for 1 h at 4°C and then fixed with acetone. Slides for total and surface fluorescence were then stained with fluorescein-conjugated goat anti-mouse immunoglobulin G for 1 h at 37°C and examined for fluorescence. Intracellular (total) immunofluorescence and surface immunofluorescence were both readily observed in recombinant-infected cells (Fig. 3A and B). Only background-level immunofluorescence was seen in cells infected with the wild-type baculovirus (Fig. 3C) or with mock-infected Sf9 cells (data not shown). Thus, the expressed gI appeared to be transported to the cell surface.

To investigate induction of neutralizing antibodies, BALB/c mice (6 to 8 weeks old) were vaccinated three times subcutaneously and intraperitoneally (concomitantly) at 3-week intervals with freeze-thawed whole insect cells expressing gI as we described previously for baculovirus-expressed gD (3). Subcutaneous injections were given with 1×10^6 cells infected with the baculovirus gI recombinant (72 h postinfection, multiplicity of infection of 10) (containing approximately 8 μg of gI) mixed with Freund's complete adjuvant on day 0 and with Freund's incomplete adjuvant on days 21 and 42. Intraperitoneal injections were given by using 1×10^6 cells, infected in a similar manner, suspended in phosphate-buffered saline on the same days. Mock-vaccinated mice were inoculated in a similar manner with Sf9 cells infected with wild-type baculovirus. A positive control group was immunized three times intraperitoneally with 2×10^5 PFU of HSV-1 strain KOS.

Mice were bled 3 weeks after the final vaccination. Pooled sera from immunized and mock-vaccinated mice were heat inactivated, diluted in minimal essential medium, mixed with 100 PFU of HSV-1, and incubated for 30 min at 37°C. Fresh or heat-inactivated guinea pig complement (2.5%) was added, and the mixture was incubated for an additional 30 min. Duplicate samples were added to CV-1 cells in 24-well microtiter plates. The plates were incubated at 37°C for 72 h and stained with 1% crystal violet, and the plaques were counted to assay residual HSV-1 infectivity. The experiment was repeated twice, and the means of the antibody titers were expressed as the reciprocal of the serum dilution. The gI neutralization titer (50% plaque reduction) was 167 in the presence of complement and 91 in the absence of complement (Table 1). Neutralizing activity therefore appeared to be partially complement dependent. These results are similar to those reported by Sullivan and Smith (17). The levels of complement-dependent and non-complement-dependent neutralizing antibodies induced in mice vaccinated with live HSV-1 (strain KOS) were significantly higher than those induced by the recombinant gI (Table 1). This was not unexpected, since immunization with whole virus allows responses to other viral components in addition to gI. A neutralizing-antibody titer of less than 10 was produced in mock (AcNPV)-vaccinated animals in the presence or absence of complement (Table 1).

To examine the ability of vaccination with the expressed gI to provide protection against lethal infection, mice vaccinated three times subcutaneously and intraperitoneally (concomitantly) as described above were challenged intraperitoneally with 2×10^6 PFU (four 50% lethal doses) of the virulent HSV-1 strain Mckrae 3 weeks after the final vaccination. Sixty-one percent of the mock-vaccinated mice died within 14 days, while 90% of mice vaccinated with expressed gI survived (Table 1). In the positive control group, 100% of mice immunized with KOS were protected (Table 1). Thus, immunization with baculovirus-expressed gI can protect mice from lethal intraperitoneal HSV-1 challenge.

We have shown here that vaccination of naive mice with recombinant gI resulted in the production of complement-dependent and non-complement-dependent neutralizing antibodies to HSV-1. This result is similar to results obtained with a recombinant vaccinia virus expressing gI (17). More interestingly, we found that immunization with recombinant gI can provide protection against HSV-1 challenge. This is in contrast to the results of Blacklaws et al. (2), who did not find any protection against virus challenge by using a gI vaccinia virus recombinant. This difference might be due to the larger amount of gI polypeptide received by our baculovirus-expressed-gI-vaccinated mice (approximately 8 μg of gI per injection) or to immunogenic differences between the expressed glycoproteins. Regardless of the reason for these differences, this is the first report to demonstrate protection against lethal HSV-1 challenge by using a gI vaccine. Thus, gI may be of value as part of a subunit vaccine for HSV-1.

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