Antiviral Research, 18 (1992) 291–302

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AVR 00561

Baculovirus expressed herpes simplex virus type 1 glycoprotein C protects mice from lethal HSV-1 infection

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(Received 10 October 1991; accepted 30 January 1992)

Summary

A recombinant baculovirus (vAc-gC1) was constructed that expresses the glycoprotein C (gC) gene of herpes simplex virus type 1 (HSV-1). When Sf9 cells were infected with this recombinant, a protein that was smaller in size than authentic HSV-1 gC was detected by Western blotting using anti-gC polyclonal antibody. The recombinant gC was susceptible to tunicamycin, partially resistant to Endo-H, and was found on the membrane of Sf9 cells. Antibodies raised in mice to recombinant gC reacted with gC from HSV-1 infected cells and neutralized the infectivity of HSV-1 in vitro. Immunized mice were protected from lethal challenge with HSV-1.

Glycoprotein C; HSV-1; Immunogenicity; Baculovirus

Introduction

Each of the ten HSV-1 glycoproteins is encoded by a different viral gene (McGeoch et al., 1988; Spear, 1985). Although the functions of each glycoprotein have not yet been completely defined, the viral glycoproteins appear to play an important role in the infectious process and in viral pathogenesis (Spear, 1985). Specific glycoproteins are involved in virus

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attachment (Fuller and Spear, 1985; Herold et al., 1991), penetration (Sarmiento et al., 1979; Fuller and Spear, 1987; Highlander et al., 1988), envelopment and egress from the infected cell, and membrane fusion (Gompels and Minson, 1986; Minson et al., 1986).

It is important to understand the role each glycoprotein may play in terms of protective immunity. gC is of particular interest because it is a receptor for the C3b component of complement (Baucke and Spear, 1979; Seidel-Dugan et al., 1988). Langeland et al. (1987) suggested that gC is involved with, but not essential for, binding to the cellular receptor. Recently, Tanner et al. (1987) postulated that gC, by analogy to EBV gP350/220, may play some role in the process of HSV-1 infection by binding to the C3b fragment of complement. Herold et al. (1991) also reported that gC may play a role in the adsorption of HSV-1 to the cell surface.

gC has been termed 'non-essential', since viruses with mutations in this gene grow normally in tissue culture (Holland et al., 1984; Homa et al., 1986b). However, this 'non-essential' designation is without regard to potential in vivo roles for gC (Friedman et al., 1986). gC is a major antigenic determinant of HSV-1 (Glorioso et al., 1984b). gC can elicit cell-mediated immunity (Rosenthal et al., 1987; Glorioso et al., 1985), and antibodies against gC can participate in complement-dependent and cell-mediated lysis of HSV-1 infected cells (Balachandran et al., 1982; Glorioso et al., 1984a). Monoclonal antibodies against gC can neutralize HSV-1 in vitro and protect animals against lethal HSV-1 challenge (Balachandran et al., 1982). Vaccinia virus expressing gC (Weir et al., 1989) has been shown to be protective in animals.

The purpose of the present study was to use the strong polyhedrin gene promoter of baculovirus in a baculovirus expression system to produce large quantities of HSV-1 gC and analyze its biochemical properties and its immunogenicity in mice. The DNA sequence encoding the complete HSV-1 glycoprotein C (gC) was inserted into a baculovirus transfer vector under control of the polyhedrin gene promoter of *Autographa californica* nuclear polyhedrosis virus (AcNPV) and recombined into baculovirus. The recombinant gC that was produced was glycosylated and was found on the membrane of Sf9 cells. Antibodies raised in mice to recombinant gC neutralized the infectivity of HSV-1 in vitro and immunized mice were protected from lethal challenge with HSV-1.

Materials and Methods

Viruses and cells. The E2 strain of *Autographa californica* nuclear polyhedrosis virus (AcNPV) and *Spodoptera frugiperda* (Sf9) cells were grown in TNM-FH media (J.R.H. Biosciences, Lenexa, KS) containing 10% fetal bovine serum as described previously (Inumaru et al., 1987).

Construction of AcNPV recombinant transfer vector. Clone pGC364 (Homa et

al., 1986a) from HSV-1 strain KOS containing a complete DNA copy of the gC gene was linearized by restriction enzyme digestion with Nhe I and the linearized plasmid was treated with Bal 31. After addition of BamH I linker, the resulting fragment was inserted into the BamH I site of the pVL941 vector (Summers and Smith, 1987). As confirmed by restriction enzyme analysis and partial sequencing, this construct contains the entire sequence of the gC gene. It has a non-coding region of 20 nucleotides in front of the first ATG. This is followed by the complete coding region of 1533 nucleotides and 403 nucleotides after the gC termination codon at the 3′ end.

Transfection and selection of recombinant viruses. Sf9 cells were co-transfected with purified infectious baculovirus (AcNPV) DNA and pAc-gC1 plasmid DNA as described (Summers and Smith, 1987). Following three cycles of plaque purification, five polyhedrin-negative recombinant viruses were selected. The five recombinant baculoviruses all expressed gC with similar properties as determined by Western blotting using anti-gC polyclonal antibody. One of the recombinants was chosen for further study and designated vAc-gC1.

Western blots. The procedures used for Western immunoblot analyses have been described previously (Ghiasi et al., 1991a).

Endoglycosidase H (Endo-H) treatment. Endo-H treatment was done on lysates from Sf9 infected cells (10 PFU/cell; 72 h post-infection) as described by the manufacturer (Boehringer Mannheim Biochemicals). Briefly, 1×10^5 cells were lysed in 20 μl of 1% *n*-octylglucoside. 180 μl of sodium acetate (50 mmol, pH 5.0), phenylmethylsulfonyl fluoride (0.5 mmol), and 20 mU of Endo-H (Boehringer Mannheim Biochemicals) were added, and the samples were incubated at 37°C overnight. At the end of the incubation period, samples were precipitated with acetone and 50 μl of sample buffer (Laemmli, 1970) was added.

Tunicamycin treatment. Infected cells (10 PFU/cell) were incubated in 4 μg/ml tunicamycin (Sigma, St Louis, MO) in TNM-FH media from 0 to 48 h post-infection.

Immunofluorescence. Sf9 cells were infected with wild-type AcNPV or recombinant gC (vAc-gC1) at an MOI of 10 PFU/cell and incubated for 72 h. To look at intracellular (total cell) fluorescence, cells were washed with PBS, fixed with acetone and incubated with rabbit antibody to gC (R46) (Eisenberg et al., 1987) for 1 h at 37°C. To examine cell surface immunofluorescence, unfixed, unpermeabilized cells were incubated with rabbit anti-gC polyclonal antibody for 1 h at 4°C and then fixed with acetone. Slides for intracellular and surface fluorescence were then stained with fluorescein-conjugated goat anti-rabbit IgG antibody for 1 h at 37°C, and examined for fluorescence.

Immunization of mice. Sf9 cells infected with wild type AcNPV or vAc-gC1 (10 PFU/cell, 72 h post-infection) were collected, washed and suspended in PBS. Mice (Balb/C, 6–8 weeks old) were vaccinated 3 times subcutaneously and intraperitoneally with whole insect cells expressing gC or whole insect cells infected with wild type AcNPV (mock vaccination). Subcutaneous injections were done using 1×10^6 cells with Freund's complete adjuvant on day 0, or with Freund's incomplete adjuvant on days 21 and 42. Intraperitoneal injections were done on the same days using 1×10^6 cells in PBS. A group of positive control mice were immunized three times intraperitoneally with 2×10^5 PFU of KOS.

ELISA and serum neutralization assays. Sera from immunized mice were collected 3 weeks after the final vaccination and pooled. Antibody titrations were performed in triplicate on the pooled sera by ELISA as described (Ghiasi et al., 1991a).

For in vitro serum neutralization assays, serial 2-fold dilutions of heat-inactivated pooled sera were made in MEM, mixed with 30 pfu of HSV-1 strain KOS and incubated for 30 min at 37°C. Fresh or heat-inactivated guinea pig complement (2.5%) was added and the mixture incubated for another 30 min. Duplicate samples were assayed for residual HSV-1 infectivity on CV-1 cells in 24-well microtiter plates. The plates were incubated at 37°C for 72 h, stained with 1% crystal violet, and plaques were counted. The neutralization titer of the serum is expressed as the reciprocal of the highest dilution showing a 50% reduction in the number of plaques compared to virus controls.

Viral challenge. Three weeks after the final vaccination, mice were challenged intraperitoneally with 4 LD50 (2×10^6 PFU) of HSV-1 (strain Mckrae) and monitored for a period of two weeks.

Results

Expression of gC in insect cells. To determine the steady-state levels of recombinant gC expression, infected Sf9 cells (10 PFU/cell) were harvested at various times post-infection and analyzed by Western blotting. The recombinant gC reacted strongly with rabbit antibody to gC (R46) producing a broad band (Fig. 1, lanes 3–6). Shorter exposure times suggested that this band may consist of two closely migrating bands (arrows). gC was readily detected at 48, 72, and 96 h post-infection (lanes 3–5). The decline at 120 h (lane 6) is coincident with the beginning of cell lysis. The anti-gC antiserum used here did not react with any proteins from wild-type baculovirus infected or uninfected Sf9 cell extracts (lanes 7 and 8).

In HSV-1 infected cells, two major forms of gC (mature gC and the partially glycosylated gC precursor, pgC) are detected (Peake et al., 1982; Spear, 1976; Compton and Courtney, 1984a; Wenske et al., 1982). In this study, pgC and gC

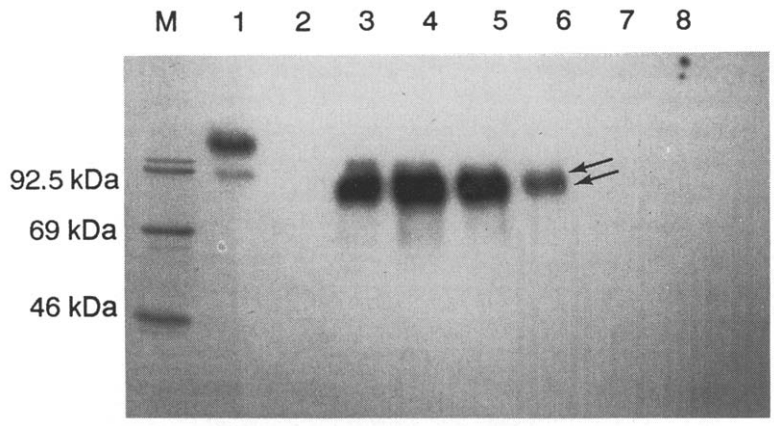

Fig. 1. Time course of the synthesis and accumulation of baculovirus expressed gC. Sf9 cells were infected with the baculovirus-gC recombinant (vAc-gC1) at an MOI of 10 PFU/cell. Vero cells were infected with HSV-1 strain KOS at an MOI of 10 PFU/cell for 20 h. 1×10^5 gC infected Sf9 cells or Vero-infected HSV-1 cells were lysed in sample buffer and analyzed by Western blots using polyclonal anti-gC antibody. Lanes: M, molecular weight markers; 1, HSV-1 infected Vero cells; 2, vAc-gC1 infected cells harvested at 24 h post-infection; 3, vAc-gC1 infected cells harvested at 48 h post-infection; 4, vAc-gC1 infected cells harvested at 72 h post-infection; 5, vAc-gC1 infected cells harvested at 96 h post-infection; 6, vAc-gC1 infected cells harvested at 120 h post-infection; 7, wild-type baculovirus-infected cells; 8, mock-infected Sf9 cells.

from HSV-1 infected Vero cells had apparent molecular weights of 90 kDa and 120 kDa respectively (Fig. 1, lane 1; also see Fig 2, A and B, lanes 1). The broad (doublet) band representative of the major baculovirus expressed gC species appeared similar in size to pgC (Fig. 1 lane 1, lower band; also compare lanes 1 with lanes 2 in Fig. 2A and Fig. 2B). Thus, the baculovirus expressed gC had an apparent molecular weight of 85–105 kDa.

Glycosylation of gC. To determine if the expressed gC was N-glycosylated, infected cells were treated with tunicamycin (an inhibitor of asparagine-linked glycosylation). Total cell extracts were analyzed by Western blotting using anti-gC polyclonal antibody. The tunicamycin treatment generated an expressed gC related polypeptide (Fig. 2A, lane 3), smaller than the untreated expressed gC (lane 2), with an apparent molecular weight of 70 kDa. This apparent molecular weight is similar to that of HSV-1 gC synthesized in vivo (Compton and Courtney, 1984b) and in vitro (Frink et al., 1983) in the presence of tunicamycin. Thus, like native gC, the untreated expressed gC appeared to contain N-linked sugars and hence be glycosylated (although the glycosylation pattern may not be identical). In HSV-1 infected cells, gC also contains O-linked sugars (Wenske and Courtney, 1983). However, Sf9 cells do not usually add O-linked glycans at serine or threonine residues (Sissom and Ellis, 1989). A lack of O-linked glycosylation may account for the 'mature' expressed gC being smaller than the HSV-1 gC.

To determine if the expressed gC contained complex sugars, recombinant gC and viral gC were treated with Endo-H (removes high mannose chains). Fig. 2B

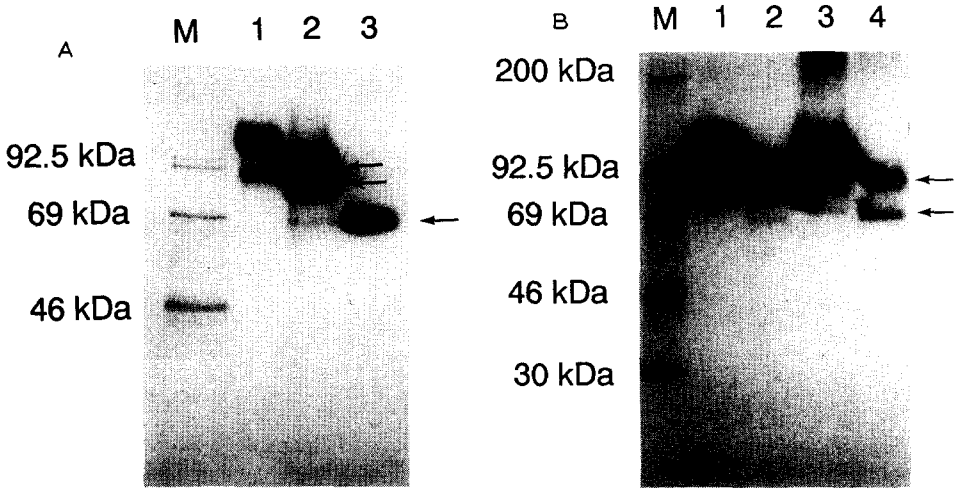

Fig. 2. Glycosylation of baculovirus expressed gC. Panel A: effect of tunicamycin on the electrophoretic mobility of gC. Insect cells were infected with the baculovirus recombinant vAc-gC1 (10 PFU/cell) in the presence of 4 μg of tunicamycin/ml of media. Total cell lysates were analyzed by Western blots using polyclonal anti-gC antibody. Lanes: M, molecular weight markers; 1, HSV-1 infected Vero cells, 2, vAc-gC1 infected cells; 3, vAc-gC1 infected cells treated with 4 μg/ml tunicamycin throughout the infection (0 48 h). Panel B: effect of endoglycosidase H treatment on baculovirus expressed gC. Lysates from baculovirus-gC recombinant (vAc-gC1) infected cells were treated with endoglycosidase H (Endo-H) as described in Materials and Methods, and analyzed by Western blots using polyclonal anti-gC antibody. Lanes: M, molecular weight markers; 1, HSV-1 infected Vero cells; 2, vAc-gC1 infected cells; 3, Endo-H treated HSV-1 infected Vero cells; 4, Endo-H treated vAc-gC1 infected cells.

shows a comparison by Western analysis of viral HSV-1 gC synthesized in Vero cells (lane 1, no Endo-H; lane 3, + Endo-H) and recombinant gC synthesized in Sf9 cells (lane 2, no Endo-H; lane 4, + Endo-H). Digestion of recombinant gC with Endo-H resulted in a new protein with an apparent molecular weight of 70 kDa (lane 4, lower arrow) similar to that seen above following tunicamycin treatment. However, a significant amount of the original band remained, apparently not affected by Endo-H treatment (lane 4, upper arrow). Endo-H treatment also generated a minor protein band of approximately 70 kDa in HSV-1 infected cells (lane 3). In addition, Endo-H treatment of HSV-1 gC appeared to most greatly affect pgC (the lower band in lane 1). These results suggest that similar to native gC (Wenske et al., 1982; Compton and Courtney, 1984a,b; Kuhn et al., 1988), the baculovirus expressed gC is a mixture of both Endo-H resistant and Endo-H susceptible sugars.

Localization of recombinant gC in insect cells. To determine whether the expressed gC was transported to the cell surface, vAc-gC1 infected Sf9 cells were examined by indirect immunofluorescence staining using anti-gC polyclonal antibody (Fig. 3). Intracellular immunofluorescence was readily observed in cells infected with the recombinant gC (panel, A). To look specifically for gC on the cell surface, indirect immunofluorescence staining was

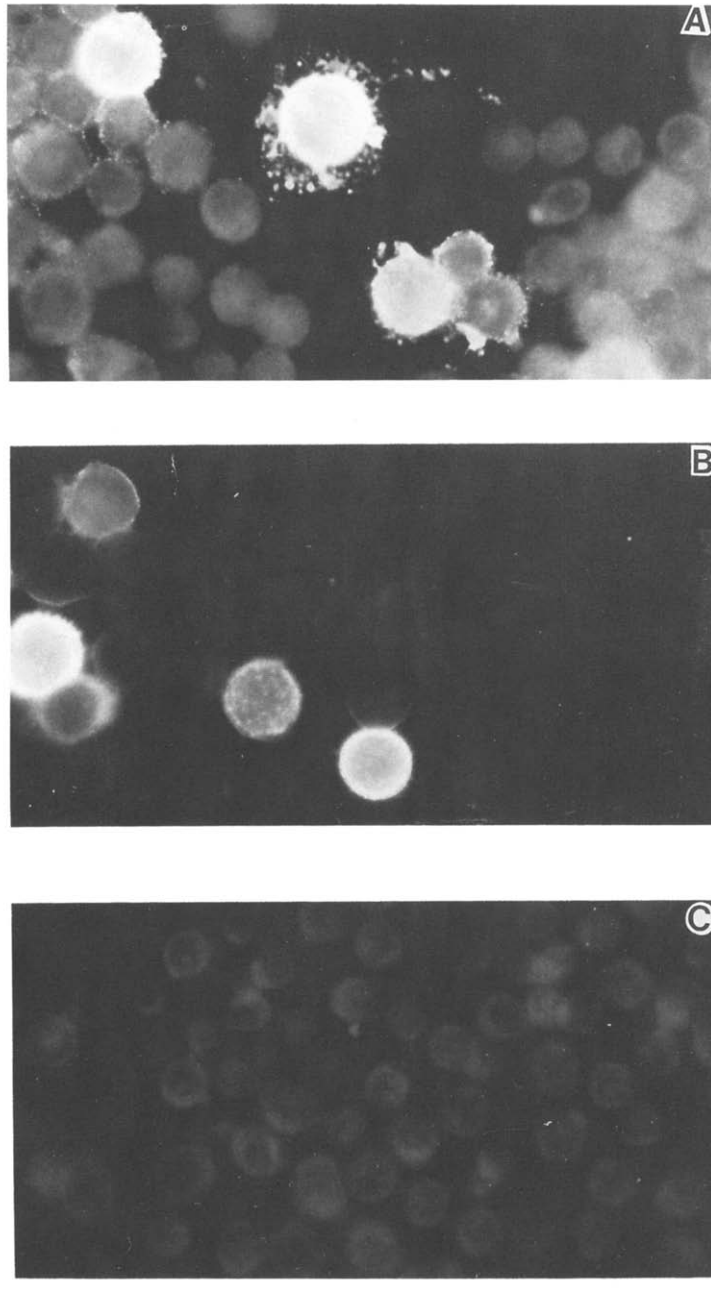

Fig. 3. Immunofluorescence of recombinant baculovirus-infected cells. Intracellular and surface indirect immunofluorescence using anti-gC polyclonal antibody followed by fluorescein-conjugated goat anti-rabbit IgG antibody was done as described in Materials and Methods. A: recombinant vAc-gC1 infected cells, intracellular (total) fluorescence; B: vAc-gC1 infected cells, surface fluorescence; C: wild-type baculovirus-infected cells, total fluorescence.

TABLE 1

HSV-1 specific ELISA and neutralization responses after immunization of mice with a baculovirus recombinant (vAc-gC1) expressing HSV-1 gC
ELISA titers and neutralization titers are expressed as reciprocal geometric means ± standard error.

Antigen	ELISA titer	Neut. Titer	
		Fresh C'	Heat inactivated C'
vAc-gC1	1200 ± 400	178 ± 34	127 ± 87
HSV-1	2400 ± 800	>320	>320
AcNPV (mock)	<50	<30	<20

done on cells prior to fixation (and permeabilization) (panel, B). The surface fluorescence on vAc-gC1 infected cells was strong. No immunofluorescence was seen in cells infected with AcNPV (wild-type baculovirus) (panel, C). Immunofluorescence studies using gC monoclonal antibody similarly showed that in HSV-1 infected cells gC is located on the nuclear envelope and cell surface (Koga et al., 1986).

Antibody response induced by recombinant gC. Balb/C mice were immunized three times subcutaneously and intraperitoneally with whole insect cells expressing gC as described in Materials and Methods. Three weeks after the final vaccination, mice were bled. Sera pooled from groups of mice immunized with the recombinant gC, HSV-1, or wild-type baculovirus (mock-vaccinated) were heat-inactivated and reacted with HSV-1 in the presence of fresh or heat-inactivated complement. Sera from recombinant gC vaccinated mice neutralized HSV-1 infectivity in vitro (Table 1). The presence of fresh complement slightly enhanced this neutralizing activity. Mice vaccinated with live HSV-1 had higher neutralization titers than gC vaccinated animals. Neutralizing antibody was low in the mock (AcNPV) vaccinated animals.

Pooled sera were also analyzed by ELISA (Table 1). vAc-gC1 vaccinated mice had lower ELISA titers than mice vaccinated with live HSV-1. This may be due to using total HSV-1 protein as the antigen for these ELISAs. Controls

TABLE 2

Immunization of mice with a baculovirus recombinant (vAc-gC1) expressing HSV-1 gC
Survival rates (protection) following i.p. challenge with HSV-1 (McKrae) (see Materials and Methods) of the baculovirus gC recombinant and KOS-vaccinated mice were significantly different from the mock-vaccinated survival rate (Fisher's Exact test, $P=0.01$).

Antigen	No. of mice	% Survival
vAc-gC1	20	100
HSV-1	11	100
AcNPV (mock)	18	39

inoculated with wild-type AcNPV infected cells had minimal ELISA titers to HSV-1.

Vaccination with baculovirus expressed gC protects mice from HSV-1 challenge. Mice were challenged by intraperitoneal injection of 4 LD50 (2×10^6 PFU) of HSV-1 (strain McKrae) 3 weeks after the third vaccination. Sixty-one percent of the mock vaccinated mice died within 14 days. 100% of mice vaccinated with expressed gC survived (Table 2). This protection was statistically significant ($P = 0.01$). 100% of mice immunized with KOS also were protected. Similar results were reported by Schrier et al. (1983) using purified gC, and Weir et al. (1989) using vaccinia expressed gC.

Discussion

A recombinant baculovirus expressing full-length HSV-1 gC in insect cells was constructed. The level of baculovirus expressed gC at 72 h post-infection was higher than the level of gC produced in HSV-1 infected Vero cells (10 pfu/cell, 20 h post-infection) (Fig. 1, compare lane 3 to lane 1). This is similar to what we have found with other baculovirus expressed HSV-1 glycoproteins (Ghiasi et al., 1991a; Ghiasi et al., 1991b).

Like other glycoproteins expressed in insect cells (Kuroda et al., 1986; Ghiasi et al., 1991a; Greenfield et al., 1988), the baculovirus expressed gC had a smaller apparent molecular weight than native HSV-1 gC. This is likely due to a lack of O-glycosylation in insect cells. gC contains 12–16 potential O-linked oligosaccharide sites (Serafini-Cessi et al., 1984; Serafini-Cessi et al., 1989; Johnson and Spear, 1983).

As judged by immunofluorescence, the baculovirus expressed gC was transported to the cell surface. Both tunicamycin and Endo-H treatments generated a new, smaller, gC-related band with an apparent molecular weight of 70 kDa, similar in size to the un-glycosylated form of gC (Compton and Courtney, 1984a; Wenske et al., 1982). This change in mobility is consistent with 8 potential N-linked gC glycosylation sites (Serafini-Cessi et al., 1984; Serafini-Cessi et al., 1989; McGeoch et al., 1988) and suggests that the expressed gC was N-glycosylated.

Vaccination of mice with expressed gC resulted in production of ELISA and neutralizing antibodies to HSV-1. In contrast to Eberle and Courtney (1980) who reported that in the absence of complement the neutralizing activity of antibody to gC was poor, the neutralizing activity of sera from mice immunized with baculovirus gC was only slightly enhanced by complement. Finally, vaccinated animals were completely protected against lethal HSV-1 infection. Similar results were reported for purified HSV-1 gC (Schrier et al., 1983) and a vaccinia virus recombinant expressing gC (Weir et al., 1989).

gC is one of the most abundant HSV-1 glycoproteins, and antibody for gC (along with that for gB) predominates in the sera of mice infected with HSV-1

(Glorioso et al., 1984b; Oakes and Lausch, 1981). The ability to produce large amounts of baculovirus expressed gC combined with its effectiveness in protecting mice against lethal HSV-1 challenge suggests that baculovirus expressed gC may be a useful candidate for subunit vaccine production.

Acknowledgements

This work was supported by the Discovery Fund for Eye Research and Public Health Service Grants EY07566, EY05939, and EY09392. Dr. Kaiwar is an Iris and B. Gerald Cantor Scholar. We thank Drs. Max Summers for pVL 941 vector, the E2 strain of AcNPV and Sf9 cells, J. C. Glorioso for the pGC364 clone, R.J. Eisenberg and G.H. Cohen for gC (R46) polyclonal antibody, and Mrs. Susan Slanina for technical assistance.

References

Balachandran, N., Bacchetti, S. and Rawls, W.E. (1982) Protection against lethal challenge of BALB/c mice by passive transfer of monoclonal antibodies to five glycoproteins of herpes simplex virus type 2. Infect. Immunol. 37, 1132–1137.

Baucke, R.B. and Spear, P.G. (1979) Membrane proteins specified by herpes simplex viruses. V. Identification of an Fc-binding glycoprotein. J. Virol. 32, 779–789.

Compton, T. and Courtney, R.J. (1984a) Virus-specific glycoproteins associated with the nuclear fraction of herpes simplex virus type 1 infected cells. J. Virol. 49, 594–597.

Compton, T. and Courtney, R.J. (1984b) Evidence for post-translational glycosylation of a non-glycosylated precursor protein of herpes simplex virus type 1. J. Virol. 52, 630–637.

Eberle, R. and Courtney, R.J. (1980) Preparation and characterization of specific antisera to individual glycoprotein antigens comprising the major glycoprotein region of herpes simplex virus type 1. J. Virol. 35, 902–917.

Eisenberg, R.J., Ponce de Leon, M., Friedman, H.M., Fries, L.F., Frank, M.M., Hastings, J.C. and Cohen, G.H. (1987) Complement component C3b binds directly to purified glycoprotein C of herpes simplex virus type 1 and 2. Microbiol. Pathogen. 3, 423–435.

Friedman, H.M., Glorioso, J.C., Cohen, G.H., Hastings, J.C., Harris, S.L. and Eisenberg, R.J. (1986) Binding of complement component C3b to glycoprotein gC of herpes simplex virus type 1: mapping of gC binding sites and demonstration of conserved C3b binding in low passage clinical isolates. J. Virol. 60, 470–475.

Frink, R.J., Eisenberg, R., Cohen, G. and Wagner, E.K. (1983) Detailed analysis of the portion of the herpes simplex virus type 1 genome encoding glycoprotein C. J. Virol. 45, 634–647.

Fuller, A.O. and Spear, P.G. (1985) Specificities of monoclonal and polyclonal antibodies that inhibit absorption of herpes simplex virus to cells and lack of inhibition by potent neutralising antibodies. J. Virol. 55, 475–482.

Fuller, A.O. and Spear, P.G. (1987) Anti-glycoprotein D antibodies that permit absorption but block infection by herpes simplex virus 1 prevent virion cell fusion at the cell surface. Proc. Natl. Acad. Sci. USA 84, 5454–5458.

Ghiasi, H., Kaiwar, R., Nesburn, A.B. and Wechsler, S.L. (1991a) Immunoselection of recombinant baculoviruses expressing high levels of biologically active herpes simplex virus type 1 glycoprotein D. Arch. Vir. 121, 163–178.

Ghiasi, H., Nesburn, A.B. and Wechsler, S.L. (1991b) Cell surface expression of herpes simplex virus type-1 glycoprotein H in recombinant baculovirus infected cells. Virology 185, 187–194.

Glorioso, J., Schroder, C.H., Kumel, G., Szczesiul, M. and Levine, M. (1984a) Immunogenicity of

herpes simplex virus glycoproteins gC and gB and their role in protective immunity. J. Virol. 50, 805–812.

Glorioso, J., Schroder, C.H., Kumel, G., Szczesiul, M. and Levine, M. (1984b) Immunogenicity of herpes simplex virus glycoproteins gC and gB and their role in protective immunity. J. Virol. 50, 805–812.

Glorioso, J., Kees, U., Kumel, G., Kirchner, H. and Krammer, P.H. (1985) Identification of herpes simplex virus type 1 (HSV-1) glycoprotein gC as the immunodominant antigen for the HSV-1 specific memory cytotoxic T lymphocytes. J. Immunol. 135, 575–582.

Gompels, U. and Minson, A. (1986) The properties and sequence of glycoprotein H of herpes simplex virus type 1. Virology 153, 230–247.

Greenfield, C., Patel, G., Clark, S., Jones, N. and Waterfield, M.D. (1988) Expression of the human EGF receptor with ligand stimulatable kinase activity in insect cells using a baculovirus vector. EMBO J. 7, 139–146.

Herold, B.C., Wudunn, D., Soltys, N. and Spear, P.G. (1991) Glycoprotein C of herpes simplex virus type 1 plays a principal role in the adsorption of virus to cells and in infectivity. J. Virol. 65, 1090–1098.

Highlander, S.L., Cai, W., Person, S., Levine, M. and Glorioso, J.C. (1988) Monoclonal antibodies define a domain on herpes simplex virus glycoprotein B involved in virus penetration. J. Virol. 62, 1881–1888.

Holland, T.C., Homa, F.L., Marlin, S.D., Levine, M. and Glorioso, J. (1984) Herpes simplex virus type 1 glycoprotein C-negative mutants exhibit multiple phenotypes, including secretion of truncated glycoproteins. J. Virol. 52, 566–574.

Homa, F.L., Otal, T.M., Glorioso, J.C. and Levine, M. (1986a) Transcriptional control signals of a herpes simplex virus type 1 late (gamma 2) gene lie within bases −34 to +124 relative to the 5′ terminus of the mRNA. Mol. Cell Biol. 6, 3652–3666.

Homa, F.L., Purifoy, D.J.M., Glorioso, J.C. and Levine, M. (1986b) Molecular basis of the glycoprotein C-negative phenotypes of herpes simplex virus type 1 mutants selected with a virus neutralizing monoclonal antibody. J. Virol. 58, 281–289.

Inumaru, S., Ghiasi, H. and Roy, P. (1987) Expression of bluetongue virus group specific antigen VP3 in insect cells by a baculovirus vector: its use for the detection of bluetongue virus antibodies. J. Gen. Virol. 68, 1627–1635.

Johnson, D.C. and Spear, P.G. (1983) O-linked oligosaccharides are acquired by herpes simplex virus glycoproteins in the golgi apparatus. Cell 32, 987–997.

Koga, J., Chatterjee, S. and Whitley, R.J. (1986) Studies on herpes simplex virus type 1 glycoproteins using monoclonal antibodies. Virology 151, 385–389.

Kuhn, J.C., Eing, B.R., Brossmer, R., Munk, K. and Braun, R.W. (1988) Removal of N-linked carbohydrates decreases the infectivity of herpes simplex virus type 1. J. Gen. Virol. 69, 2847–2858.

Kuroda, K., Hauser, C., Rott, R., Klenk, H.D. and Doerfler, W. (1986) Expression of the influenza virus haemagglutinin in insect cells by a baculovirus vector. EMBO J. 5, 1359–1365.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.

Langeland, N., Holmsen, H., Lillehaug, J.R. and Haarr, L. (1987) Evidence that neomycin inhibits binding of herpes simplex virus type 1 to the cellular receptor. J. Virol. 61, 3388–3393.

Luckow, V.A. and Summers, M.D. (1988) Trends in the development of baculovirus expression vectors. BioTechnology 6, 47–54.

McGeoch, D.J., Dalrymple, M.B., Davison, A.J., Dolan, A., Frame, M.C. McNab, D., Perry, L.J., Scott, J.E. and Taylor, P. (1988) The complete DNA sequence of the long unique region in the genome of herpes simplex virus type 1. J. Gen. Virol. 69, 1531–1574.

Minson, A.C., Hodgman, T.C., Digard, P., Hancock, D.C., Bell, S.E. and Buckmaster, E.A. (1986) An analysis of the biological properties of monoclonal antibodies against glycoprotein D of herpes simplex virus and identification of amino acid substitutions that confer resistance to neutralisation. J. Gen. Virol. 67, 1001–1013.

Oakes, J.E. and Lausch, R.N. (1981) Role of Fc fragments in antibody-mediated recovery from

ocular and subcutaneous herpes simplex virus infections. Infect. Immun. 33, 109–114.
Peake, M.L., Nystrom, P. and Pizer, L.I. (1982) Herpes virus glycoprotein synthesis and insertion into plasma membranes. J. Virol. 42, 678–690.
Rosenthal, K.L., Smiley, J.R., South, S. and Johnson, D.C. (1987) Cells expressing herpes simplex virus glycoprotein gC but not gB, gD, or gE are recognized by murine virus specific cytotoxic T-lymphocytes. J. Virol. 61, 2438–2447.
Sarmiento, M., Haffey, M. and Spear, P.G. (1979) Membrane proteins specified by herpes simplex virus. III. Role of glycoprotein VP7 (B2) in virion infectivity. J. Virol. 29, 1149–1158.
Schrier, R.D., Pizer, L.I. and Moorehead, J.W. (1983) Type-specific delayed hypersensitivity and protective immunity induced by isolated herpes simplex virus glycoprotein. J. Immunol. 130, 1413–1418.
Seidel-Dugan, C., Ponce de Leon, M., Friedman, H.M., Fries, L.F., Frank, M.M., Cohen, G.H. and Eisenberg, R.J. (1988) C3b receptor activity on transfected cells expressing glycoprotein C of herpes simplex virus types 1 and 2. J. Virol. 62, 4027–4036.
Serafini-Cessi, F., Dall'Olio, F., Pereira, L. and Campadelli-Fiume, G. (1984) Processing of N-linked oligosaccharides from precursor to mature form herpes simplex virus type 1 glycoprotein gC. J. Virol. 51, 838–844.
Serafini-Cessi, F., Dall'Olio, F., Malagolini, N. and Campadelli-Fiume, G. (1989) Temporal aspects of O-glycosylation of glycoprotein C from herpes simplex virus type 1. Biochem. J. 262, 479–484.
Sissom, J. and Ellis, L. (1989) Secretion of the extracellular domain of the human insulin receptor from insect cells by use of a baculovirus vector. Biochem. J. 261, 119–126.
Spear, P.G. (1976) Membrane proteins specified by herpes simplex viruses. I. Identification of four glycoprotein precursors and their products in type 1-infected cells. J. Virol. 17, 911–1008.
Spear, P.G. (1985) Glycoproteins specified by herpes simplex virus. In: B. Roizman (Ed.), The herpes viruses, Vol. 3, pp. 315–356. Plenum Press, New York.
Summers, M.D. and Smith, G.E. (1987) A manual of methods for baculovirus vectors and insect cell culture procedures, Texas Agricultural Experimental Station Bulletin no. 1555.
Tanner, J., Weis, J., Fearon, D., Whang, Y. and Kieff, E. (1987) Epstein-Barr virus gp350/220 binding to the B lymphocyte C3d receptor mediates adsorption, capping and endocytosis. Cell 50, 203–213.
Weir, J.P., Bennett, M., Allen, E.M., Elkins, K.L., Martin, S. and Rouse, B.T. (1989) Recombinant vaccinia virus expressing the herpes simplex virus type 1 glycoprotein C protects mice against herpes simplex virus change. J. Gen. Virol. 70, 2587–2594.
Wenske, E.A., Bratton, M.W. and Courtney, R.J. (1982) Endo-beta-*n*-acetylglucosaminidase H sensitivity of precursors to herpes simplex virus type 1 glycoproteins gB and gC. J. Virol. 44, 241–248.
Wenske, E.A. and Courtney, R.J. (1983) Glycosylation of herpes simplex virus type 1 gC in the presence of tunicamycin. J. Virol. 46, 297–301.