Induction of protective immunity with antibody to herpes simplex virus type 1 glycoprotein H (gH) and analysis of the immune response to gH expressed in recombinant vaccinia virus

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Introduction

Glycoprotein H (gH) of herpes simplex virus type 1 (HSV-1) is one of three glycoproteins that are essential for virus viability in tissue culture (Cai et al., 1988; Ligas & Johnson, 1988; Desai et al., 1988), and homologues of this glycoprotein have been identified in members of all the herpesvirus subfamilies (Davison & Taylor, 1987; Gompels et al., 1988; Cranage et al., 1988; Heineman et al., 1988). gH is required for virus entry (Desai et al., 1988) and is probably involved in cell to cell spread of infectivity since gH-specific monoclonal antibodies (MAbs), in addition to neutralizing free virus, inhibit cell fusion by syncytial strains and prevent intercellular virus transmission (Buckmaster et al., 1984; Gompels & Minson, 1986). Antibodies raised against the varicella-zoster virus and Epstein-Barr virus homologues have similar properties implying functional conservation (Keller et al., 1987; Miller & Hutt-Fletcher, 1988).

The antiviral properties of gH-specific MAbs suggest that a good humoral response to gH might be of value in modifying HSV infection in vivo. Most studies of immune responses to HSV have emphasized the importance of cell-mediated immunity (reviewed by Nash et al., 1985), and in at least one infection model, animals lacking functional B lymphocytes have been shown to recover normally from HSV infection (Simmons & Nash, 1987). Despite the undoubted importance of cell-mediated responses in recovery from HSV infection, there are nevertheless ample data that demonstrate the ability of antibody to protect animals from HSV challenge by several different routes. Thus several studies have shown that passive antibody, administered before or soon after infection, is protective (e.g. Simmons & Nash, 1985; Balanchandran et al., 1982). It is uncertain whether antibody acts directly by neutralizing virus and lysing infected cells, or acts indirectly by increasing the efficiency with which inflammatory cells are recruited to the primary infection site, but it appears that protection is best achieved by active immunization with glycoproteins that are major neutralizing targets or by passive administration of antibodies directed against these targets (e.g. Long et al., 1984; Martin et al., 1989; Blacklaws et al., 1990; Balanchandran, 1982).

The objective of the work described in this paper was to examine the protective effect of passive immunization
with anti-gH antibody or active immunization with recombinant vaccinia viruses expressing HSV-1 gH. We found that gH-specific antibody protected efficiently but that vaccination with recombinant vaccinia viruses failed to elicit neutralizing antibody against HSV-1 and did not protect against challenge with HSV-1. This apparent contradiction was explained by examination of gH expressed by recombinant vaccinia viruses, which revealed that the protein was antigenically distinct from that found in HSV-1, a result analogous to that obtained when HSV-1 gH was expressed in Cos-1 cells (Gompels & Minson, 1989). Coexpression of gH with either gD or gB failed to rescue the authentic antigenic form of gH. Thus, although antibody against HSV-1 is protective, an effective means of delivering immunogenic gH is not yet available.

Methods

Cells and viruses. BHK-21 cells were grown in Glasgow modified Eagle's medium supplemented with 10% tryptose phosphate broth and 10% newborn bovine serum (ETC). CV-1 cells and 143 thymidine kinase-deficient (TK-) cells were grown in the same medium supplemented with 10% foetal bovine serum. HSV-1 strains SC16 and HFEM were propagated and assayed in BHK-21 cells. Vaccinia virus strain WR and recombinant viruses derived from it were propagated in BHK-21 cells and assayed in CV-1 cells.

Antibodies. MAbs antibodies LP11, 52S and 53S are specific for HSV-1 gB (Buckmaster et al., 1984; Showalter et al., 1981). MAbs AP7 and LP2 are specific for HSV gD (Minson et al., 1986). Ascites fluids used for passive immunizations contained 3 to 5 mg/ml IgG. Anti-trpE-gH is a rabbit serum raised against a tryptophan E-HSV-1 gH fusion protein (Desai et al., 1988). Neutralization assays were performed as described by Minson et al. (1986). Preparation of infected cell lysates, immunoprecipitation, detection of enzymes by Western blotting and immunofluorescence were as described by Gompels & Minson (1989).

Animal models. All experiments were done with 4- to 6-week-old female BALB/c mice. Two HSV infection models were used. The ear model, in which inoculum is introduced subdermally into the ear pinna is described by Hill et al. (1975). The flank or zosteriform model in which inoculum is scarified into the depilated flank epidermis and forms a zosteriform lesion following spread via the sensory nerves is described by Sydskis & Schultz (1965) and by Simmons & Nash (1984). Mice were vaccinated by intraperitoneal (i.p.) injection with 10^7 p.f.u. of recombinant vaccinia virus in 0.1 ml phosphate-buffered saline and were re-vaccinated with the same dose by the same route 10 to 14 days later. HSV challenge infections were performed 7 days later. Passive antibody was introduced as ascites fluid via the tail vein. Virus titres in the infected ear during the acute phase of infection were determined by homogenizing the ear pinnae and storing the homogenate at -70°C on the inoculated (left) side of the animal. The pooled ganglia were then incubated for 5 days in ETC, homogenized and assayed for infectivity. Appearance of plaques was taken to indicate establishment of latent infection.

Recombinant vaccinia viruses. Recombinant vaccinia viruses VII and VgDS2 (Cantin et al., 1987; Cremer et al., 1985) express HSV-1 gB and gD, respectively and were kindly provided by the authors. Vac YC3 was used as a negative control for vaccination and expresses a schistosome surface antigen (G. Smith, unpublished results). All three recombinants contain the foreign insert within the TK coding sequence and are TK-. The recombinant viruses expressing the HSV-1 gH were constructed using the general strategy described by Mackett et al. (1984) as follows. The gH coding sequence (open reading frame UL22) of HSV-1 strain HFEM (Gompels & Minson, 1988) was isolated on a 3.5 kb NcoI-Xhol fragment from a NcoI/partial Xhol digest (nucleotides 46382 to 42879; McGeoch et al., 1988), in which the initiating ATG lay within the NcoI site. This fragment was end-repaired and inserted into the Smal site of pSP64. The coding sequence was then reisolated by digestion with HindIII and Xhol and was inserted into HindIII/Smal-digested pGS62 (Cranage et al., 1986) such that the gH coding sequence under the control of the vaccinia virus 7.5K early/late promoter was inserted within the vaccinia virus TK gene. An analogous construct was made in which the gH coding sequence was placed under the control of the vaccinia virus 4b late promoter (Rosel & Moss, 1985) by insertion into pRK19, an insertion vector similar to pGS62 except that the 7.5K promoter sequences are replaced by the 4b promoter (R. K. Kent & G. L. Smith, unpublished results). Before being inserted into pRK19 the HindIII/Xhol gH coding fragment was transferred from pSP64 to M13mp18 and was modified by site-directed mutagenesis (Kunkel, 1985) such that the sequence immediately 5' of the initiating ATG was replaced with the sequence TAAATG, the motif found in most vaccinia virus late mRNAs (Rosel et al., 1986). gH coding sequences in pGS62 and pRK19 were introduced into vaccinia virus by transfection and selection for TK-recombinants as described by Mackett et al. (1984). Recombinant vaccinia viruses in which gH was expressed under the control of the 7.5K or the 4b promoter were named Vac7.5-gH and Vac4b-gH respectively.

Results

Recombinant vaccinia viruses

Vaccinia virus recombinants were constructed in which the HSV-1 gH coding sequence was placed under the control of either the vaccinia virus 7.5K promoter (Vac7.5-gH) or the vaccinia virus 4b promoter (Vac4b-gH). BHK-21 cells were infected with recombinant viruses, harvested at various times over a 16 h period and lysates were examined by Western blotting. The results (Fig. 1) show that cells infected with either recombinant accumulate larger amounts of gH than cells infected with HSV-1, that gH synthesized by recombinant vaccinia viruses appears to have an electrophoretic mobility intermediate between that of the immature and mature forms of gH found in HSV-1-infected cells and that gH expressed by the different recombinants is qualitatively indistinguishable, but expression from the 4b promoter is greater than that from the 7.5K promoter. The 4b promoter is active only after viral DNA synthesis and drives the expression of a major virion core component (Moss & Rosenblum, 1973; Rosel & Moss, 1985). The 7.5K promoter is active both before and after viral DNA synthesis (Cochran et al., 1985; Mackett et al., 1984). The absence of gH synthesis at early times in cells infected with Vac7.5-gH is
Immunity to HSV-1 gH

Fig. 1. Western blot analysis of gH expressed by recombinant vaccinia viruses. Lysates were prepared at 2 h (lanes 1 and 2), 4 h (lanes 3 and 4), 6 h (lanes 5 and 6), 8 h (lanes 7 and 8), 16 h (lanes 9, 10 and 11) post-infection (p.i.) from BHK-21 cells infected with Vac7.5-gH (lanes 1, 3, 5, 7 and 9), Vac4b-gH (lanes 2, 4, 6, 8 and 10) or HSV-1 strain HFEM (lane 11). The samples were then electrophoresed through a 7.5% polyacrylamide gel, transferred to nitrocellulose and reacted with trpE-HSV-1 gH fusion antiserum. Cells were infected with recombinant vaccinia viruses at an m.o.i. of 20 and with HSV-1 HFEM at an m.o.i. of 10. All lanes were loaded with extracts prepared from approximately 5 x 10^6 cells.

Table 1. Effect of passive immunization of mice with antibodies on zosteriform spread

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Target</th>
<th>Number of mice</th>
<th>Zosteriform spread</th>
</tr>
</thead>
<tbody>
<tr>
<td>LP11</td>
<td>gH</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>LP2</td>
<td>gH</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>AP7</td>
<td>gD</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>Immune serum</td>
<td></td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Control ascites</td>
<td></td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

* (-), Not done.

Table 2. Effect of immunization with vaccinia recombinants on development of zosteriform lesions and establishment of latency

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of mice</th>
<th>Zosteriform spread</th>
<th>Latent infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vac7.5-gH</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Vac4b-gH</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>VgD52</td>
<td>5</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>YC3</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

probably due to the presence of the sequence T_{6}CT in the gH coding sequence, since the motif T_{5}NT is a transcription termination signal in ‘early’ vaccinia virus messages (Rohrmann et al., 1986).

Passive immunization

Several studies have demonstrated the ability of passively administered MAb or immune sera against HSV to protect against virus challenge but antibodies to HSV-1 gH have not been tested in this way. Before proceeding with active immunization of animals with recombinant vaccinia viruses, we wished to establish that a humoral response to gH would have a protective effect. Groups of mice were inoculated by flank scarification with 5 x 10^6 p.f.u. of HSV-1 strain SC16 and after 24 h were injected intravenously with 100 µl of ascitic fluid containing MAbs specific for gD or gH or with 1 ml of an HSV-1-immune mouse serum. Although the monoclonal IgG content of each ascites fluid was not determined, all ascites contained 3 to 5 mg/ml of IgG. In addition ascites fluids containing antibodies LP2 (gD-specific) and LP11 (gH-specific), both of which neutralize HSV-1 in the absence of complement, gave similar dilution endpoints in neutralization tests (>=1:2000, <1:4000). Control animals received an ascites fluid containing an irrelevant MAb and all such animals developed zosteriform lesions 5 or 6 days after infection. All animals were examined daily until 10 days after infection and the results (Table 1) show that MAb to gH was solidly protective. The efficacy of anti-gD antibodies confirmed previous findings (Simmons & Nash 1987). Although this is a relatively superficial examination of the effects of passive gH antibody on the infection process, it nevertheless serves to establish that a good humoral response to gH should modulate virus infection and suggests that gH has potential as a protective immunogen.

Immunization with recombinant vaccinia virus

The efficacy of vaccination with recombinants expressing gH was examined by comparison with recombinants expressing gD or with an irrelevant recombinant. Mice were given two vaccinations with 10^7 p.f.u. of recombinant vaccinia virus by the i.p. route and were challenged with 10^5 p.f.u. of HSV-1 strain SC16 by flank scarification or with 5 x 10^4 p.f.u. of SC16 by subdermal inoculation in the ear pinna. The vaccinia virus recombinant expressing gD protected all mice from zosteriform spread of challenge virus and only one of five animals became latently infected (Table 2), a result in agreement with previous findings (Cremer et al., 1985). By comparison, vaccination with Vac7.5-gH or Vac4b-gH did not modify the challenge infection. Mice challenged by the ear pinna route were killed 5 days after challenge and the virus titre at the challenge site was determined. Only one of five animals vaccinated with the gD recombinant yielded detectable challenge virus from the ear pinna (Fig. 2) while yields of virus from animals vaccinated with Vac7.5-gH or Vac4b-gH were not significantly different to those from control mice.
Fig. 2. Virus clearance from the ear pinnae of mice immunized with recombinant vaccinia viruses. Groups of five mice were injected twice i.p. with a 14 day interval with 10^7 p.f.u. of VgD52, Vac7.5-gH, Vac4b-gH or YC3 then challenged with 5 x 10^4 p.f.u. of HSV-1 SC16 in the left ear pinna. The ear pinnae were removed 5 days after challenge and remaining virus titred by plaque assay on BHK-21 cells. The results have been expressed as the p.f.u. of virus in each pinnae.

Fig. 3. Neutralization activity against HSV-1 of sera from mice immunized with recombinant vaccinia viruses. Dilutions of sera pooled from mice injected with VgD52 (■), YC3 (○), Vac4b-gH (■) or Vac7.5-gH (●) were incubated with 5 x 10^3 p.f.u. of HSV-1 SC16 in the presence of complement for 1 h at room temperature. Remaining viable virus was then titrated in duplicate on BHK-21 cells and the mean plaque number is shown. The endpoints are expressed as the reciprocal of the highest dilution of antiserum that gave a 50% reduction in plaque number.

Pooled sera from parallel groups of vaccinated mice were tested for neutralizing activity against HSV-1. Sera from mice vaccinated with the gD recombinant contained neutralizing antibody with an endpoint of approximately 1:1024, while sera from animals vaccinated with Vac7.5-gH or Vac4b-gH contained little, if any, neutralizing activity (Fig. 3). Thus neither gH recombinant was effective in inducing neutralizing antibody or in modulating the outcome of challenge infection by the routes used.

Properties of gH synthesized by vaccinia virus recombinants

The failure of recombinant vaccinia virus expressing gH to elicit neutralizing antibody is surprising in view of the high levels of expression achieved by comparison with HSV-1-infected cells and the fact that most MAbs to gH have neutralizing activity (Showalter et al., 1981; Buckmaster et al., 1984). These considerations suggested that the form of gH expressed by recombinant vaccinia virus might be different from the authentic molecule expressed by HSV-1, a suggestion reinforced by the slight difference in electrophoretic mobilities shown in Fig. 1. The antigenic characteristics of gH expressed by HSV-1 and by vaccinia virus recombinants were investigated using three MAbs specific for gH. Antibodies 52S, 53S and LP11, all of which react with conformation-dependent epitopes, were used to precipitate gH from infected cell lysates. After gel electrophoresis and transfer to nitrocellulose the precipitation products were detected using a polyclonal antiserum to a trpE-gH fusion protein. The results (Fig. 4) show that all three antibodies precipitated gH from lysates of HSV-1-infected cells but only antibody 52S precipitated gH from lysates of cells infected with the recombinant vaccinia viruses. Immunofluorescence of BHK-21 cells infected with HSV-1 or with vaccinia virus recombinants (Fig. 5)
showed that antibody 52S detected gH on the surface and in the cytoplasm of HSV-1-infected cells, but no surface staining was apparent on cells infected with Vac7-5-gH or Vac4b-gH. Staining was limited to the cytoplasm and the nuclear membrane in these cells. It appears that when gH is synthesized by recombinant vaccinia viruses the molecule is antigenically aberrant and is not expressed at the cell surface, but it is unlikely that these abnormalities are due to some adverse effect of vaccinia virus replication on gH synthesis because similar findings were reported by Gompels & Minson (1989) using gH expression plasmids in the temperature-sensitive Cos cell system. In the latter study it was found that the authentic form of gH could be rescued by superinfecting virus, as a result that was interpreted as indicating that the correct synthesis and processing of gH is dependent on other HSV-1-specific functions acting in trans. The finding that aberrant gH is formed when expressed alone in two quite different expression systems supports this interpretation. We have no idea as to the nature of the HSV-specific functions required for authentic gH synthesis, but one obvious possibility is that gH must interact with a second viral glycoprotein. Since, apart from gH, only gB and gD are essential for HSV-1 replication in vitro, any postulated obligatory interaction of a virus-specific glycoprotein with gH must involve either gB or gD. To test this possibility BHK-21 cells were infected with Vac4b-gH either alone or in combination with VII (expressing gB) or VgD52 (expressing gD), each at an m.o.i. of 20. After 16 h the cellular localization of gH was examined by immunofluorescence with antibody 52S and the antigenicity of gH was determined by immunoprecipitation with antibodies 52S, 53S and L-P11. The results were similar to those shown in Fig. 4 and 5; the cellular localization and antigenic characteristics of gH expressed by Vac4b-gH were not modified by coexpression of gB or gD.

**Discussion**

Although gH is a minor component of the HSV-1 envelope, its conservation among all members of the herpesvirus group examined to date implies a central role for this molecule in the infection process. Yet we know little of the function of gH or of the immune response to it, other than that antibodies specific for gH have notable antiviral effects on the virus particle and on the infected cell. Nothing is known of the antibody response to gH in individuals seropositive for HSV, but it seems unlikely that human sera contain high levels of anti-gH antibody because the HSV-1 neutralizing titres of human sera correlate well with anti-gD titres implying that gD antibodies account for most of the neutralizing activity (Cranage et al., 1983). Since it remains impossible to synthesize gH in an authentic antigenic form in the absence of other HSV proteins, direct and reliable measurement of anti-gH antibodies cannot be achieved.

The neutralizing and anti-fusion properties of gH-specific antibodies are similar to those of some antibodies against gD (Minson et al., 1986; Gompels & Minson, 1986; Noble et al., 1983), an antigen that has long been recognized as providing protective immunity in experimental animals both by active and passive immunization (Long et al., 1984; Cremer et al., 1985; Berman et al., 1985; Blacklaws et al., 1987; Krishna et al., 1989; Balachandran et al., 1982; Simmons & Nash, 1985). By analogy we might expect gH to be protective and we have demonstrated that LP11, a MAb specific for HSV-1 gH, provides efficient protection against zosteriform spread of HSV-1 in the mouse. Active immunization with gH expressed by a vaccinia virus recombinant vector failed either to protect or to elicit neutralizing antibody. This is explained in part by the altered antigenic form of gH expressed in this system. However, at least one neutralizing epitope is present on the
recombinant gH because the molecule is recognized by 52S, a neutralizing MAAb. The failure of gH synthesized by vaccinia virus recombinants to be expressed at the infected cell surface may contribute to its failure as an immunogen.

The demonstration that gH when synthesized in two quite different expression systems is antigenically abnormal strengthens the conclusion of Gompels & Minson (1989) that the correct synthesis and processing of gH is dependent on other HSV-specific function(s). Our experiments suggest that neither of the other essential HSV glycoproteins, gB and gD, is involved, although this interpretation of the results should be qualified by the recognition that the vaccinia virus-infected cell might be an unsuitable environment for the relevant interactions to occur. It is generally assumed that the expression of individual virus glycoprotein genes in mammalian vectors results in the synthesis of an authentic protein, but it is clear that this is not always the case. For example, synthesis of the correct conformational form of influenza virus haemagglutinin requires a functional M2 protein (Hay et al., 1985; Belshe et al., 1988). We have no idea of the requirements for the authentic synthesis of gH, but until this can be achieved, studies of the functions of gH or of the immune response to it will be severely hampered. Finally, we do not know whether the problems we have encountered in expressing HSV-1 gH are associated with an HSV-specific phenomenon, or whether the homologues of gH in other herpesviruses have similar characteristics. However, it has been noted that expression of the gH homologue of human cytomegalovirus (the product of the UL75 open reading frame; Chee et al., 1990) in recombinant vaccinia virus results in failure of cell surface expression (Cranage et al., 1988).

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References


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