Analysis of protective immune responses to the glycoprotein H–glycoprotein L complex of herpes simplex virus type 1

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A recombinant vaccinia virus expressing both glycoprotein H (gH) and glycoprotein L (gL) of herpes simplex virus type 1 (HSV-1) was used to examine the protective response to gH–gL in immunized mice and to compare these responses with those induced by the highly protective immunogen, glycoprotein D (gD). Weak levels of HSV-1-specific neutralizing antibody were obtained in response to the gH–gL complex, virus clearance from the site of challenge was marginally enhanced compared to that observed following immunization with gH alone, and gH–gL was found to protect mice against acute infection in the ganglia, although not as efficiently as gD.

Glycoprotein H (gH) is conserved in all members of the herpesvirus family (Gompels et al., 1988; Davison & Taylor, 1987; Cranage et al., 1988; Heineman et al., 1988; Klupp & Mettenleiter, 1991) and is known to play an essential role in virus entry, based on the neutralizing and anti-fusion properties of gH-specific monoclonal antibodies (Gompels & Minson, 1986; Fuller et al., 1989; Miller & Hutt-Fletcher, 1988; Keller et al., 1987) together with the phenotypic characterization of gH-deficient mutant viruses (Desai et al., 1988; Forrester et al., 1992). It has also been demonstrated that passive immunization of mice with the HSV-1 gH-specific monoclonal antibody LP11 provided protection from HSV-1-induced zosteriform spread at levels equivalent to those elicited by antibodies to glycoprotein D (gD) (Simmons & Nash, 1985; Forrester et al., 1991), and that some protection is provided by immunization with a purified preparation of gH (Chan et al., 1985). These observations, together with the finding that gH expressed by baculovirus induces neutralizing antibody in mice (Ghiasi et al., 1992) have led to the prediction that despite its low abundance in the virion, humoral responses to gH may play a role in modifying herpesvirus infection in vivo.

The expression of individual glycoprotein genes of HSV-1 in vaccinia virus recombinants has enabled a number of groups to assess their relative importance as targets of the immune system, and has confirmed that gD, and glycoproteins B (gB) and C (gC) induce high titres of virus-neutralizing antibody and good levels of protection against HSV-1 challenge infection in animals (Cremer et al., 1985; Cantin et al., 1987; Martin et al., 1989; Weir et al., 1989; Blacklaws et al., 1990). Glycoprotein H, however, when expressed in vaccinia virus and examined for immunogenicity in mice, failed to elicit neutralizing antibodies or to induce protection against HSV-1 challenge (Blacklaws et al., 1990; Forrester et al., 1991) although in these experiments the gH molecule expressed was aberrantly processed and not transported to the cell surface (Forrester et al., 1991). This seems to be a general feature of herpesvirus gH molecules when expressed in mammalian cells in the absence of other virus proteins (Gompels & Minson, 1989; Foa-Tomasi et al., 1991; Cranage et al., 1988).

It has subsequently been shown (Hutchinson et al., 1992) that authentic folding and surface expression of HSV-1 gH requires association with another essential HSV glycoprotein, gL, and that a positional homologue of gL performs an analogous role in the processing of human cytomegalovirus (HCMV) gH (Kaye et al., 1992). In the light of these findings, we therefore aimed to re-evaluate the immunogenicity of gH when expressed in the presence of gL by constructing a vaccinia virus recombinant expressing both gH and gL of HSV-1.

The insertion vector pSC11 (Chakrabarti et al., 1985), which contains two vaccinia virus promoters, p11 and p7.5, was used as the starting plasmid for obtaining co-expression of HSV-1 gH and gL in vaccinia virus. The gL gene from HSV-1 strain 17 had previously been isolated as an 825 bp fragment (nucleotides 9329 to 10154) and was obtained as an end-repaired BamHI–EcoRI fragment from the plasmid pRKUL1 (Hutchinson et al., 1992). This was cloned into the SmaI site of pSC11 downstream of the p7.5 early/late promoter. The BamHI fragment of pSC11 containing the two vaccinia virus promoters was modified by site-specific mutagenesis (Kunkel et al., 1987) so as to remove the ATG initiation
codon of the late vaccinia virus 11K gene and to conserve the TAAAT consensus sequence of late vaccinia virus promoters. These modifications were carried out using the synthetic oligonucleotide 5’ CTATGCTATAAATA-AGCTTTCTCGAGG 3’. Additional restriction endonuclease sites were introduced at the BamHI site immediately 5’ of the gL coding sequence using a synthetic oligonucleotide linker containing the restriction sites BglII/SmaI/BglII//BamHI/BglII, and this allowed insertion of the modified promoters as a BamHI fragment and insertion of the gH coding sequence (of HSV-1 strain HFEM) as a BglII fragment, derived as described previously for the construction of the recombinant virus Vac4bgH (Forrester et al., 1991). The essential features of the final plasmid used to produce recombinant virus gHLvacc are shown in Fig. 1 (a). Since it had been documented by others that gD expressed by vaccinia virus induced a high degree of protection in vivo, we also constructed a recombinant vaccinia virus (gDvacc) expressing HSV-1 gD under control of the 4b late vaccinia virus promoter, to use as a positive control. The gD coding sequence of HSV-1 strain Patton was obtained as an end-repaired HindIII–NruI fragment. The HindIII restriction site lies 74 base pairs 5′ of the initiation codon of the gD gene, and NruI cuts 161 base pairs 3′ of the gD termination codon. This blunt-ended fragment was cloned downstream of the 4b vaccinia virus late promoter at the SmaI site of pRK19, and thymidine kinase-negative recombinants were obtained following the established transfection and selection methods described by Mackett et al. (1984).

Before examining the ability of gH–gL to elicit protective immune responses to HSV-1 in vivo it was necessary to establish that the gH molecule expressed by this construct was antigenically authentic, as determined by recognition by the monoclonal antibody LP11, since formation of the LP11 epitope has previously been shown to require co-expression of gH and gL (Hutchinson et al., 1992). It was also important to compare the level of gH expression from gHLvacc with that in HSV-1-infected cells. BHK21 cells were infected at 10 p.f.u./cell for 18 h with HSV-1 strain HFEM, Vac4bgH, Vac4bgH plus Vac4bgL, or with gHLvacc, and cell lysates were immunoprecipitated either with monoclonal antibody 52S (Showalter et al., 1981) which recognizes both gL-associated and unassociated forms of gH, or with LP11. After electrophoresis of samples corresponding to 10^6 infected cells, immunoprecipitates were transferred to nitrocellulose and reacted with polyclonal anti-trpE-HSV-1 gH serum (Desai et al., 1988). Fig. 1 (b) shows that the level of expression of gH in gHLvacc-infected cells is similar to that in HSV-1-infected cells and to that in co-infected cells in which gH and gL are expressed from the 4b promoter. Furthermore, the gH molecule produced in gHLvacc-infected cells appears to be processed and folded correctly, since it is recognized by both 52S and LP11 antibodies, and immunofluorescence studies confirmed expression of gH at the cell surface (data not shown).

To assess the immunogenicity of the gH–gL complex, 4 to 6 week old female BALB/c mice were immunized intraperitoneally with 10^7 p.f.u./mouse of recombinant vaccinia virus and were boosted as above, 14 days later. Sera were collected 7 days after boosting, and the mice were then challenged with 5 x 10^4 p.f.u. of HSV-1 SC16 scarified onto the left ear pinna. Ear pinnae (experiment 1 and experiment 2) and cervical ganglia (CII to CIV) (experiment 2) were removed 5 days after HSV-1 challenge and, following homogenization, virus titres in these tissues were determined by plaque assay on BHK21 cells. Pooled sera from groups of six mice were tested for
neutralizing activity against HSV-1, both in the presence and absence of guinea-pig complement. Fig. 2 shows that sera from mice immunized with gDvacc contained complement-independent neutralizing antibodies with an end-point of 1/256. End-points are expressed as the reciprocal dilution of sera giving a 50% reduction in plaque numbers. This is in agreement with the findings of others using similar immunization protocols (Blacklaws et al., 1990; Forrester et al., 1991). The gHLvacc-infected mice produced much lower levels of neutralizing serum antibody than did gDvacc-infected animals but, nevertheless, weak complement-independent neutralizing activity with an end-point of approximately 1/16 was observed. Immunization with Vac4bgH or Vac4bgL alone failed to induce measurable levels of neutralizing antibody, either in the presence or absence of complement, as did the control vaccinia virus recombinant, US27.

The comparative effects of vaccination with gD and gH–gL on clearance of challenge HSV-1 infection were determined by measuring virus titres during acute infection following inoculation of HSV-1 onto the ears of immunized mice, and the results from two separate experiments are shown in Table 1. In both experiments, immunization with gDvacc induced protective response both at the site of challenge and even more markedly in the sensory ganglia. The ear pinnae of gDvacc-immunized mice showed a 3 to 3.5 log₁₀ reduction in virus titres compared with the control groups and no virus was recovered from the cervical ganglia of any of these animals, in agreement with the studies of others who have shown that immunization with gD is extremely effective at inducing virus clearance in a number of different systems, an effect thought to be mediated both by antibody and by CD4⁺ T lymphocyte responses (Eisenberg et al., 1985; Berman et al., 1985; Krishna et al., 1989). Immunization with either gLvacc or gHvacc failed to protect any of the animals from HSV-1 infection, with virus titres in the ears and ganglia showing little difference from the control groups. This is perhaps not surprising since both gH and gL are incorrectly processed when expressed alone in vaccinia virus and are not presented on the cell surface. In both experiments vaccination with the gHLvacc recombinant afforded limited protection against HSV-1 challenge in the ear pinnae of mice, with virus titres showing approximately 0.7 to 1.5 log₁₀ reductions compared with the control groups. A more dramatic protective effect was seen in the ganglia, where vaccination with gHLvacc led to considerable reductions in virus titres in all animals.

Previous attempts to determine the immunogenicity of HSV-1 gH expressed by vaccinia virus have been hampered by the failure to obtain expression of the molecule in an antigenically authentic form. The vaccinia virus recombinant, gHLvacc, described in this report and which expresses fully processed gH has enabled us to examine the protective effects of the gH–gL complex in the mouse model. This reagent may also prove useful for evaluating the extent of humoral responses to gH in HSV-1 seropositive individuals. Although monoclonal antibodies to gH have potent neutralizing activity in vitro and can protect animals from HSV-1 infection if administered passively, it is clear from the results presented here that gH–gL expressed in vaccinia virus is not capable of inducing high levels of neutralizing antibody and affords only minor protection against acute infection at the site of virus challenge. Similar observations were made when gH produced by baculovirus was used as an immunogen (Ghiasi et al., 1992).
and found to induce complement-independent neutralizing antibody but not to protect against lethal HSV-1 challenge. The extent to which neutralizing antibodies against HSV-1 glycoproteins contribute towards protective effects in vivo is somewhat unclear; cell-mediated immunity is certainly important in recovery from HSV infections (Nash et al., 1985) and both gB and gC induce virus-specific cytotoxic T cells (McLaughlin-Taylor et al., 1988; Glorioso et al., 1985), while gD is a target for CD4+ lymphocytes. However, the low levels of neutralizing antibody induced by gHL vacc may have been sufficient to limit spread of virus to the sensory ganglia. Nevertheless, the effects of gH–gL immunization were considerably less potent than might have been predicted from the in vitro properties of gH-specific antibodies, suggesting that the gH–gL complex is unlikely to contribute greatly towards protective responses to herpesviruses in vivo.

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References


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