Baculovirus-expressed glycoprotein H of herpes simplex virus type 1 (HSV-1) induces neutralizing antibody and delayed type hypersensitivity responses, but does not protect immunized mice against lethal HSV-1 challenge

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We have shown previously that herpes simplex virus type 1 (HSV-1) glycoprotein H (gH) expressed by a baculovirus recombinant is transported to the cell surface in the absence of other HSV-1 gene products, and that the expressed gH has an apparent Mr similar to that of authentic HSV-1 gH. We report here that antibodies raised in mice to this baculovirus-expressed gH neutralize the infectivity of HSV-1 in vitro; this neutralizing activity was not complement-dependent. Mice vaccinated with gH also developed delayed type hypersensitivity (DTH) to HSV-1. This is the first report of expressed HSV-1 gH inducing neutralizing antibody or DTH responses in vaccinated animals. In contrast to the gH expressed in mammalian systems, the ability of this baculovirus-expressed gH to induce a neutralizing antibody response may be due to the inability of the mammalian expression system to transport gH to the cell surface. Despite inducing anti-HSV-1 neutralizing antibody and DTH responses, vaccination of mice with gH did not protect the mice against lethal intraperitoneal challenge with HSV-1.

Monoclonal antibodies (MAbs) to glycoprotein H (gH) of herpes simplex virus type 1 (HSV-1) neutralize HSV-1 in a complement-independent manner (Showalter et al., 1981; Buckmaster et al., 1984; Gompels & Minson, 1986). Anti-gH MAbs neutralize HSV-1 efficiently and can inhibit plaque formation when added to cell monolayers shortly after infection (Gompels & Minson, 1986). Anti-gH MAbs do not inhibit adsorption of virus to cells, but appear to neutralize HSV-1 by blocking virus penetration (Fuller et al., 1989).

Passive immunization of mice with anti-gH MAbs LP11 and LP2 can produce clearance of HSV-1 from the ear pinnae (Forrester et al., 1991). In contrast, mice vaccinated with two vaccinia virus recombinants expressing gH are not protected against HSV-1 zosteriform spread or the establishment of latent HSV-1 infection following virus challenge. Since these vaccinia virus recombinants also do not induce any neutralizing antibody (Forrester et al., 1991; Blacklaws et al., 1990), the lack of in vivo protection may be due to a poor immunological response to the gHs expressed. This in turn could be because these vaccinia virus-expressed gHs are smaller than authentic gH and are not transported to the cell surface (Blacklaws et al., 1990; Forrester et al., 1991).

The gHs expressed in COS cells (Gompels & Minson, 1989) and in stable cell lines (Foa-Tomasi et al., 1991) also have a lower Mr than authentic gH and are not transported to the cell surface. Recently, we have expressed the gH gene of HSV-1 using a high level baculovirus [Autographa californica nuclear polyhedrosis virus (AcNPV)] expression system (Ghiasi et al., 1991c). This recombinant gH is transported to the cell surface and has an Mr similar to that of authentic HSV-1 gH (Ghiasi et al., 1991c). Therefore, it was of particular interest to determine whether this baculovirus-expressed gH could induce a neutralizing antibody response and whether it could protect mice against lethal challenge with HSV-1.

To study the induction of neutralizing antibodies by our baculovirus-expressed gH, 35 BALB/c mice (6 to 8 weeks old) were vaccinated subcutaneously and intraperitoneally (i.p.) (concomitantly) with frozen and thawed whole Sf9 insect cells expressing gH as described previously (Ghiasi et al., 1991b). Subcutaneous injections contained $1 \times 10^6$ cells infected with the baculovirus–HSV-1 gH recombinant (72 h post-infection, m.o.i. 10) mixed with Freund's complete adjuvant; intraperitoneal injections were given using $1 \times 10^6$ cells infected similarly and suspended in PBS on the same
al., complement-independent neutralization (Showalter 1986). In contrast to our results, Blacklaws et al. (1990) have reported that mice vaccinated with two vaccinia virus–HSV-1 gH recombinants do not produce neutralizing antibodies. This difference may be due to the differences in the dose of gH polypeptide received by the vaccinated mice or to differences in the structure or intracellular (rather than surface) location of gH expressed in mammalian systems (Compels & Minson, 1989; Forrester et al., 1991; Foa-Tomas 1991).

Neutralizing antibody titres in sera from mice vaccinated three times at 3-week intervals were also examined (see below); no increase in neutralizing titres was seen (data not shown). The level of complement-dependent and complement-independent neutralizing antibodies induced in mice vaccinated with live HSV-1 (KOS) was significantly higher than that induced by the recombinant virus (Fig. 1 and Table 1). This was not unexpected because immunization with whole virus allows responses to other virus components in addition to gH. The neutralizing antibody titre in mock (AcNPV)-vaccinated mice (Fig. 1 and Table 1) was <1:10.

To study the delayed type hypersensitivity (DTH) response to gH, mice were vaccinated three times as described below. The dorsal side of one ear of each mouse was injected with 2 × 10^6 p.f.u. of u.v.-inactivated HSV-1 (strain McKrae) in 5 µl of MEM. The DTH reaction was measured before, and 24 h, 48 h and 72 h after injection using a micrometer (Mitutoyo) and recorded as net swelling (post-challenge minus pre-challenge ear thickness) (Nash et al., 1980). Controls included HSV-1-vaccinated mice (positive control) and wt baculovirus (mock)-vaccinated mice (negative control) (Table 1). Both recombinant and HSV-1 KOS-vaccinated mice developed DTH responses. Ear swelling increased from 24 h to 72 h post-challenge (Table 1), followed by a decline in swelling after day 3 (not shown). The swelling in recombinant virus-vaccinated mice was as great as that in HSV-1-vaccinated mice. Significantly less swelling was observed in mock-vaccinated (wt baculovirus) animals.

To examine the ability of vaccination with the baculovirus-expressed gH to provide protection against lethal infection, the mice vaccinated once subcuta-
neously and i.p. (concomitantly) as described above, were challenged i.p. with $2 \times 10^5$ p.f.u. of virulent HSV-1 strain McKrae 3 weeks after vaccination; the mice were monitored for 2 weeks. All of the 20 mock-vaccinated mice (100%,) and 33 of 35 (95%,) of mice vaccinated with the recombinant virus died following HSV-1 challenge. In contrast, all 15 HSV-1-vaccinated mice survived (Table 2, one vaccination). These results suggested that a single inoculation with recombinant virus has no significant effect on the susceptibility of the mice to HSV-1.

To determine whether multiple vaccinations might result in increased survival, a second set of experiments was done. BALB/c mice were vaccinated three times subcutaneously and i.p. at 3-week intervals. Subcutaneous injections were performed with Freund’s complete adjuvant on day 0 and with Freund’s incomplete adjuvant on days 21 and 42. Intraperitoneal injections were performed on the same days using PBS. Material for vaccination was prepared as described above for the single vaccine experiment. Mock-vaccinated mice were inoculated similarly with Sf9 cells infected with wt baculovirus. A positive control group was immunized three times i.p. with $2 \times 10^5$ p.f.u. HSV-1 KOS.

Again, no difference was seen between the mock- and recombinant virus-vaccinated mice, even with repeated inoculations. Of 19 recombinant virus-vaccinated mice, 11 died, and 11 of 18 mock-vaccinated mice died (Table 2, three vaccinations). As expected, 100% of the mice immunized with HSV-1 KOS were protected. Our results show that the level of protection induced by the baculovirus-expressed gH was statistically different from that induced by HSV-1 KOS ($P < 0.01$), whereas it was similar to the mock-vaccinated group. In other words, vaccination with baculovirus-expressed gH did not increase survival following challenge with a lethal dose of HSV-1.

Forrester et al. (1991) and Blacklaws et al. (1990) have reported that recombinant vaccinia viruses expressing gH do not induce detectable neutralizing antibody. In contrast, we show here that vaccination of mice with our baculovirus-expressed gH induced both neutralizing antibodies and a DTH response. However, despite these immune responses survival rates of mice challenged with a lethal dose of HSV-1 were not increased. In parallel experiments, we have obtained significant protection against lethal HSV-1 challenge using baculovirus recombinants expressing gB or gD (Ghiasi et al., 1991a, b). Interestingly, the DTH response reported here for gH was greater than the DTH responses we obtained using gB and gD in parallel experiments (H. Ghiasi et al., unpublished results). Therefore our results suggest that in mice the immune response to gH may not play a significant role in protection against lethal intraperitoneal challenge with HSV-1.

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References


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