Natural infection with herpes simplex virus type 1 (HSV-1) induces humoral and T cell responses to the HSV-1 glycoprotein H:L complex

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The glycoproteins of herpes simplex virus type 1 (HSV-1) are important targets for the immune system in the control of HSV-1 infections. The humoral and T cell responses to the glycoprotein (g)Ht(His) :gL complex of HSV-1 were studied in seven HSV-1-seropositive and three HSV-1-seronegative healthy adults. In addition, responses to HSV-1 gD, were determined. As antigens, purified soluble recombinant forms of the gHt(His) :gL complex produced by insect cells and of gD, produced by yeast cells were used. In contrast to seronegative donors, sera of all seropositive donors contained gHt(His) :gL-specific IgG. Using peripheral blood (PB) T cells, gHt(His) :gL-specific proliferative T cell responses were detected in all seropositive donors. Culture supernatants of PB T cells stimulated with recombinant gHt(His) :gL contained high levels of interferon-γ and no detectable interleukin-4, indicating their Th1 phenotype. These results show that naturally acquired HSV-1 infection induces gH:gL-specific humoral and T cell responses.

Herpes simplex viruses (HSV) are the causative agents of localized skin infections of the oral, ocular, neural and genital regions. In persons whose immune function is compromised, severe and often disseminated HSV infections are observed. At least 11 glycoproteins are encoded by HSV-1. Of these, glycoproteins H and L (gH and gL) are present as a heterodimer in the viral envelope and plasma membranes of HSV-1-infected cells (Hutchinson et al., 1992). The HSV-1 gH:gL complex has an essential function in the fusion of the viral envelope with the plasma membrane and in the cell-to-cell spread of virions (Fuller & Lee, 1992; Forrester et al., 1992). The formation of a hetero-dimer between gH and gL is essential for correct folding and processing of gH (Roop et al., 1993).

Of the HSV-1 glycoproteins, gD has been studied most extensively with respect to immune responses and immunological properties. The role of HSV gH:gL as a possible target of the immune system has been studied less extensively. It is known that monoclonal antibodies (MAbs) to the gH:gL complex can inhibit HSV-1 infections in vitro (Buckmaster et al., 1984; Showalter et al., 1981). Passive administration of neutralizing antibodies raised against the gH:gL complex protects mice from zosteriform spread of HSV-1 infections (Forrester et al., 1991; Simmons & Nash, 1985). However, immunization studies with recombinant forms of gH and gL (Ghiasi et al., 1992, 1994a, b) or recombinant vaccinia viruses expressing gH, gL and gH:gL (Browne et al., 1993; Forrester et al., 1991) induced only limited protection. Subsequently, mice immunized with recombinant complexes consisting of truncated gH and full-length gL produced by a mammalian cell line were shown to be protected from a lethal HSV-1 challenge (Peng et al., 1998).

This latter finding encouraged detailed investigations of immune responses to the HSV-1 gH:gL complex in naturally infected humans. In the present study, a soluble purified recombinant form of the HSV-1 gH:gL complex, produced and secreted by insect cells, was used to analyse the humoral and peripheral blood (PB) T cell responses in seven HSV-1-seropositive and three HSV-1-seronegative healthy volunteers. The seven HSV-seropositive donors had been naturally infected by HSV-1 and most likely not by HSV-2, since none of the sera reacted with recombinant gG2 fragments and gG2 peptides (Oda-Ikoma et al., 1998).

The recombinant complex, designated gHt(His) :gL, consisted of full-length gL and truncated gH. The gH molecule

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purified by gel filtration (Superose 6 column; Pharmacia). BioWhittaker) was harvested. The gH
was truncated before the transmembrane region at amino acid 791 and was tagged with the peptide RSHHHHHH at the C
terminus. High Five insect cells (Invitrogen) were infected with recombinant baculoviruses containing the open reading frames
of both gH_{t(His)} and gL under control of polyhedrin promoters. After 72 h of infection, the culture medium (Insect Xpress,
BioWhittaker) was harvested. The gH_{t(His)}:gL complex was isolated in a one-step purification from the culture medium by
immobilized metal affinity chromatography (D. F. Westra, unpublished). The purified gH_{t(His)}:gL complex was obtained
with a high degree of purity, as analysed on SDS-polyacrylamide gels with subsequent silver staining (Fig. 1A). The predicted molecular masses of gH_{t(His)}:gL and gD_{t} are 100, 30 and 47 kDa. Western blots with the appropriate antibodies confirmed the identity of the bands seen on the silver-stained gel (Fig. 1B–D). Size exclusion HPLC (data not shown) showed that the gH_{t(His)}:gL complex has a molecular mass of 125 kDa, and confirmed that the complex is a hetero-dimer. The purified gH_{t(His)}:gL was recognized by MAb LP11, as assayed by ELISA. Reactivity to LP11 is seen as indicative of correctly
incubated with peroxidase-conjugated goat anti-mouse (B) or goat anti-
rabbit (C, D) antibodies. Antibody binding was visualized by a luminogetic reaction. Marker proteins with molecular masses of 97, 70, 45 and
29 kDa are indicated.

In parallel, the humoral and PB T cell responses to soluble recombinant HSV-1 gD were analysed. The gD_{t} antigen, truncated at residue 314, was produced in the methylotrophic yeast Pichia pastoris. Recombinant gD_{t} was purified from the yeast medium by a two-step procedure. First, recombinant gD_{t} was separated by anion exchange chromatography (Resource Q column; Pharmacia) and, subsequently, gD_{t} was further purified by gel filtration (Superose 6 column; Pharmacia). Recombinant HSV-1 gD_{t} was seen as at least three diffuse polypeptide bands (Fig. 1), most likely due to heterogeneous glycosylation and proteolysis of gD_{t} in yeast cell cultures, and was recognized by gD-specific MAbs (data not shown).

The humoral responses to a crude HSV-1 lysate, and to recombinants gH_{t(His)}:gL and gD_{t} were analysed by ELISA. For the preparation of HSV antigen, Vero cells were infected with HSV strain McIntyre (ATCC VR-539) at an m.o.i. of 10. The infected cells were lysed by freeze–thawing followed by heat-inactivation at 65 °C for 1 h. As a control, lysed mock-infected Vero cells were used. The antigens were stored in aliquots at –80 °C. The presence of specific IgG in human sera was analysed by ELISA. A lysate of HSV-1-infected Vero cells and recombinant proteins gH_{t(His)}:gL and gD_{t} were coated overnight in 50 mM sodium bicarbonate buffer pH 9.6. Serial dilutions of the sera were incubated for 1 h. After washing, horseradish peroxidase-conjugated rabbit anti-human IgG antibodies (Dako) were added. The substrate O-phenylene-diamine.HCl was used and colour development was measured. The antibody levels were defined as the reciprocal of the serum dilution which gave an A_{405} of 0.5.

Seven HSV-1-seropositive individuals (donors 1–7) and three HSV-1-seronegative individuals (donors 8–10) were tested for IgG levels specific for HSV antigen and for gH_{t(His)}:gL complex and gD_{t} (Table 1). Six out of seven HSV-1-seropositive individuals had identical antibody levels to the gH_{t(His)}:gL complex. None of the HSV-1-seronegative donors had significant IgG levels to the antigens tested. One seropositive individual (donor 1) had a relatively low antibody level to recombinant gH_{t(His)}:gL. This response was, however, still higher than those of the seronegative donors. The antibody levels to gD_{t} varied to a larger extent. Two donors (donors 5 and 6) had both very high gD_{t}-specific antibody and very high HSV-specific antibody levels. In general, higher gD_{t}-specific antibody levels correlated with higher HSV-specific antibody levels. This correlation was not seen for the antibody levels specific for the gH_{t(His)}:gL complex. In three out of seven HSV-seropositive individuals, the gH_{t(His)}:gL antibody levels were higher than the antibody levels for gD_{t} (Table 1). The data suggest that the HSV gH:gL complex is an important antigen to which humoral immune responses are elicited upon natural HSV infection.

Peng et al. (1998) showed that sera from mice and rabbits which were immunized with correctly folded recombinant HSV-1 gH:gL complex had antibodies which neutralized HSV-1 in vitro. However, the possible protective role of these neutralizing antibodies in vivo was not addressed in this study. Passive administration of an HSV-1 gH:gL-specific MAb has been shown to protect mice from zosteriform HSV-1 infection (Simmons & Nash, 1985). To date, homologues of the gH:gL complex have been found in all herpesviruses investigated. In human donors naturally infected with the herpesvirus human cytomegalovirus (CMV), CMV gH has been identified as a major target antigen of the CMV-specific neutralizing anti-
bodies (Urban et al., 1996). Consequently, HSV-1 gH:gL-

![Fig. 1. Recombinant antigens HSV-1 gH_{t(His)}:gL and gD_{t}. Purified gH_{t(His)}:gL (A, C, D) and gD_{t} (A, B) were separated by 12.5% SDS-PAGE. Proteins were visualized by silver staining (A) and by Western blotting (B–D). For Western blotting, the proteins were transferred onto PVDF membranes and subsequently incubated with gD-specific MAb A-16 (B), with gH-specific polyclonal antibody anti-gH1 (rabbit 83) and with gL-specific polyclonal antibody W192S5. Membranes were subsequently incubated with peroxidase-conjugated goat anti-mouse (B) or goat anti-rabbit (C, D) antibodies. Antibody binding was visualized by a luminogetic reaction. Marker proteins with molecular masses of 97, 70, 45 and 29 kDa are indicated.](image-url)
Humoral and T cell responses to HSV-1 gH:gL

Table 1. Serum IgG antibody levels and PB T cell responses to crude HSV-1/Vero lysate and recombinant proteins HSV-1 gH(t(His)) :gL and gDl.

<table>
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<th>Donor</th>
<th>HSV-1</th>
<th>gH(t(His)) :gL</th>
<th>gDl</th>
<th>Vero/mock [³H]Thymidine incorporation (SI)†</th>
<th>Vero/HSV-1</th>
<th>Medium [³H]Thymidine incorporation (SI)†</th>
<th>gH(t(His)) :gL</th>
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* Antibody levels were determined by ELISA. Purified recombinants gH(t(His)) :gL and gDl and lysates of HSV-1-infected Vero cells were used as antigens. The antibody levels were defined as the reciprocal of the serum dilution which gave an A% of 0–5.

† Proliferative responses of 10⁵ PBMC against lysates of mock- (Vero/mock) and HSV-1-infected Vero cells (Vero/HSV-1), purified recombinants gH(t(His)) :gL (20 µg/ml), gDl (20 µg/ml) and medium control (medium) were measured in a 6 day lymphocyte proliferation assay by [³H]thymidine incorporation for the final 16 h. Data presented are means of triplicate cultures in counts per minute (c.p.m.) and the stimulation indices (SI) are given.

Table 2. Concentrations of gamma interferon (IFN-γ) and interleukin 4 (IL-4) in culture supernatants of antigen-stimulated PBMC.

Cytokine concentrations (pg/ml) were measured by ELISA in the culture supernatants of PBMC cultures after a 6 day stimulation with a crude lysate of HSV-1-infected Vero cells (Vero/HSV-1) and 20 µg/ml of purified recombinants HSV-1gH(t(His)) :gL and gDl respectively. The detection limits of the ELISA for IFN-γ and IL-4 were 5 pg/ml.

<table>
<thead>
<tr>
<th>Donor</th>
<th>Vero/HSV-1</th>
<th>Medium</th>
<th>gH(t(His)) :gL</th>
<th>gDl</th>
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specific antibodies may have an important role in controlling HSV-1 infection.

Proliferative responses of freshly isolated peripheral blood mononuclear cells (PBMC) to lysates of HSV-1-infected Vero cells and to recombinants gH(t(His)) :gL and gDl were examined in a lymphocyte proliferation assay. PBMC were isolated by density centrifugation of heparinized PB on a Ficoll gradient (Lymphoprep; Nycomed) and were cultured in RPMI 1640 supplemented with 10% heat-inactivated human serum. 10⁵ PBMC per well were incubated with recombinant gDl (1, 5, 10
and 20 \( \mu g/ml \), recombinant \( gH_{1[H]}:gL \) (1, 5, 10 and 20 \( \mu g/ml \)) and lysates of mock-infected and HSV-1-infected Vero cells. T cell proliferation was measured as \( [\text{H}] \) thymidine incorporation over the last 16 h of a 6 day culture period. The stimulation index (SI) for HSV was calculated as the quotient of c.p.m. from cultures stimulated with a lysate of HSV-1-infected Vero cells to c.p.m. from cultures stimulated with a lysate of uninfected Vero cells. The SI for \( gH_{1[H]}:gL \) and \( gD_1 \) was calculated as the quotient of c.p.m. from cultures stimulated with the purified glycoproteins to c.p.m. of unstimulated cultures.

PBMC of seven seropositive and three seronegative healthy adult donors were tested on two or more occasions with the antigens. Data from a representative experiment are shown in Table 1. The PB T cells of all HSV-seropositive donors (donors 1–7) responded to lysates of HSV-1-infected Vero cells, recombinant HSV-1 \( gH_{1[H]}:gL \) and \( gD_1 \). Among the three seronegative donors, two donors did not respond to the HSV antigens and donor 9 demonstrated profound PB T cell responses to the two recombinant HSV-1 glycoproteins with only a marginal response to Vero/HSV-1 lysate. The data indicate that following a natural HSV-1 infection, HSV-1 gD but also \( gH:gL \)-specific T cell immunity is elicited. Given the nature of the antigen, e.g. exogenous antigen, the antigen-specific T cell responses measured \textit{in vitro} were most likely orchestrated by CD4\(^+\) T cells.

T helper cells can be divided into subsets, Th1 and Th2, based on their production of distinct patterns of cytokines. Typical Th1 and Th2 cytokines are interferon (IFN)-\( \gamma \) and interleukin (IL)-4, respectively (Biron, 1994). To determine the Th subset of the antigen-specific PB T cells, the secretion of IFN-\( \gamma \) and IL-4 by antigen-stimulated PB T cells of the HSV-seronegative and -seropositive donors was measured (Table 2). After antigenic stimulation for 6 days, cell-free culture supernatants were assayed for IFN-\( \gamma \) and IL-4 with commercial ELISA kits as described by Verjans et al. (1998b). PB T cell cultures from donors 1–7 stimulated with HSV-1 lysate, \( gD_1 \), and \( gH_{1[H]}:gL \) secreted large amounts of IFN-\( \gamma \) and no detectable IL-4 (Table 2). Interestingly, PB T cells from donor 9 also secreted significant amounts of IFN-\( \gamma \) upon stimulation with HSV-1 antigen and recombinant \( gH_{1[H]}:gL \) and, to a lesser extent, \( gD_1 \) (Table 2). These results indicated, as described previously, that the HSV-1-specific PB T cell response is Th1-like (Ghiasi et al., 1992; Biron, 1994; Carmack et al., 1996; Cher & Mosmann, 1987; Hendricks et al., 1992).

The HSV-1 glycoproteins \( gB \), \( gC \) and \( gD \) have been identified as major targets for cytolytic HSV-1-specific CD4\(^+\) T cells (Mikloska & Cunningham, 1998). In that study, HLA-DR-expressing human epidermal keratinocytes, infected with recombinant vaccinia virus expressing \( gB \), \( gC \), \( gD \) or \( gH \), were used as target cells. The highest cytotoxicity was measured for \( gD \), followed by \( gB \) or \( gC \), and then by \( gH \). Benina et al. (1995) analysed the T cell responses to CMV; three out of five CMV-specific T cell lines, mainly consisting of CD4\(^+\)CD8\(^−\) T cells, responded to CMV gH (Beninga et al., 1996). Our study indicates that naturally acquired HSV-1 infection elicits a gH:gL-specific Th1 response. All HSV-seropositive donors responded to \( gH_{1[H]}:gL \) in the proliferation assay. Two of the three seronegative donors were negative. The result of the proliferation studies with the PB T cells of HSV-seronegative donor 9 is puzzling. Despite the PB T cell response against \( gD_1 \) and \( gH_{1[H]}:gL \) complex, the donor was still HSV-seronegative 1 year after the proliferation assays. The proliferative response to \( gH_{1[H]}:gL \) could have been caused by PB T cells which recognize peptides of the gH:gL complex of other human herpesviruses. Parts of the gH:gL complex are conserved among herpesviruses. However, the \( gD_1 \)-specific proliferative response cannot be explained by such a homology because, except for HSV-2, there are no homologues of gD in other human herpesviruses. We cannot exclude the possibility that, despite the absence of contaminating proteins present on the silver-stained SDS-gel, the relatively low PB T responses of donor 9 to \( gH_{1[H]}:gL \) and \( gD_1 \) might be directed against contaminating proteins present in very low amounts in the antigen preparations.

The role of T cell immunity in controlling systemic and local HSV-1 infections has been studied extensively. HSV-specific CD4\(^+\) and CD8\(^+\) cells with cytotoxic and/or proliferating activities are present in high frequencies in PB cells and herpetic lesions (Verjans et al., 1998a, b; Schmid, 1988; Posavad et al., 1996; Carmack et al., 1996; Koelle et al., 1994). The role of CD8\(^+\) T cells may be limited because HSV-infected cells have a reduced surface expression of MHC class I molecules (Hill et al., 1995; York et al., 1994). Nonetheless, CD8\(^+\) lymphocytes may have an important function in later stages of herpetic lesions. This is because IFN-\( \gamma \)-secreted by stimulated CD4\(^+\) Th1 cells upregulates MHC class I expression on HSV-infected cells (Posavad et al., 1998). Alternatively, some cells may be less susceptible to the HSV-induced downregulation of MHC class I (Posavad et al., 1996).

The observation that a natural HSV-1 infection elicits both a T cell (Th1-like) and humoral response to the HSV-1 gH:gL complex may be of importance for the development of a subunit vaccine. Taking into account the finding that immunization with soluble recombinant HSV-1 gH:gL protects mice from a lethal HSV-1 challenge (Peng et al., 1998), the soluble gH:gL complex may be an important candidate as a component of an effective subunit vaccine for the prevention and/or control of HSV infections.

References


Humoral and T cell responses to HSV-1 gH:gL


