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 gDt were determined. As antigens, purified soluble recombinant forms of the gHt(His):gL complex produced by insect cells and of gDt 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...
Recombinant HSV-1 gD purified by gel filtration (Superose 6 column; Pharmacia) was harvested. After 72 h of infection, the culture medium (Insect Xpress, BioWhittaker) was harvested. The gH was separated by anion exchange chromatography (Resource Q column; Pharmacia) and, subsequently, gD 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 gHt(His) and gL under control of polyhedrin promoters. After 72 h of infection, the culture medium (Insect Xpress, BioWhittaker) was harvested. The gHt(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 gHt(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 gHt(His):gL and gD, 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. 1A). Size exclusion HPLC (data not shown) showed that the gHt(His):gL complex has a molecular mass of 125 kDa, and confirmed that the complex is a hetero-dimer. The purified gHt(His):gL was recognized by MAb LP11, as assayed by ELISA. Reactivity to LP11 is seen as indicative of correctly folded gH:gL since this antibody recognizes gH only when coexpressed with gL (Hutchinson et al., 1992).

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

The humoral responses to a crude HSV-1 lysate, and to recombinants gHt(His):gL and gD, 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 gHt(His):gL and gD, 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 A490 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 gHt(His):gL complex and gD, (Table 1). Six out of seven HSV-1-seropositive individuals had identical antibody levels to the gHt(His):gL complex. None of the HSV-1-seropositive donors had significant IgG levels to the antigens tested. One seropositive individual (donor 1) had a relatively low antibody level to recombinant gHt(His):gL. This response was, however, still higher than those of the seronegative donors. The antibody levels to gD, varied to a larger extent. Two donors (donors 5 and 6) both had very high gD,-specific antibody and very high HSV-specific antibody levels. In general, higher gD,-specific antibody levels correlated with higher HSV-specific antibody levels. This correlation was not seen for the antibody levels specific for the gHt(His):gL complex. In three out of seven HSV-seropositive individuals, the gHt(His):gL antibody levels were higher than the antibody levels for gD, (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 gHt:gL complex had antibodies which neutralized HSV-1 in vivo. However, the possible protective role of these neutralizing antibodies in vitro 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 antibodies (Urban et al., 1996). Consequently, HSV-1 gH:gL-
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 gD\(_t\) 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. 10\(^5\) PBMC per well were incubated with recombinant gD\(_t\) (1, 5, 10

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### 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 gD\(_t\)

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<th>gH(_{t(His)}) :gL</th>
<th>gD(_t)</th>
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<th>Vero/HSV-1 c.p.m.</th>
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* Antibody levels were determined by ELISA. Purified recombinants gH\(_{t(His)}\) :gL and gD\(_t\) 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\(_{50}\) of 0–5.

† Proliferative responses of 10\(^5\) 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), gD\(_t\) (20 µg/ml) and medium control (medium) were measured in a 6 day lymphocyte proliferation assay by [\(^3\)H]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-\(\gamma\)) 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-1 gH\(_{t(His)}\) :gL and gD\(_t\), respectively. The detection limits of the ELISA for IFN-\(\gamma\) and IL-4 were 5 pg/ml.

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<tr>
<th>Donor</th>
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<th>Medium</th>
<th>gH(_{t(His)}) :gL</th>
<th>gD(_t)</th>
<th>Vero/HSV-1</th>
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and 20 µg/ml), recombinant gH\(_{t(His)}\)-gL (1, 5, 10 and 20 µg/ml) and lysates of mock-infected and HSV-1-infected Vero cells. T cell proliferation was measured as \[^{3}H\]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 lystate of HSV-1-infected Vero cells to c.p.m. from cultures stimulated with a lystate of uninfected Vero cells. The SI for gH\(_{t(His)}\)-gL and gD\(_{t}\) 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\(_{t(His)}\)-gL and gD\(_{t}\). 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 lystate. The data indicate that following a natural HSV-1 infection, HSV-1 gD but also gH\(_{t}\)-gL-specific T cell immunity is elicited. Given the nature of the antigen, e.g. exogenous antigen, the antigen-specific T cell responses measured 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)-γ and interleukin (IL)-4, respectively (Biron, 1994). To determine the Th subset of the antigen-specific PB T cells, the secretion of IFN-γ 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-γ 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 lystate, gD\(_{t}\), and gH\(_{t(His)}\)-gL secreted large amounts of IFN-γ and no detectable IL-4 (Table 2). Interestingly, PB T cells from donor 9 also secreted significant amounts of IFN-γ upon stimulation with HSV-1 antigen and recombinant gH\(_{t(His)}\)-gL and, to a lesser extent, gD\(_{t}\) (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. Beninga 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\(_{t}\)-gL-specific Th1 response. All HSV-seropositive donors responded to gH\(_{t(His)}\)-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\(_{t}\) and gH\(_{t(His)}\)-gL complex, the donor was still HSV-seronegative 1 year after the proliferation assays. The proliferative response to gH\(_{t(His)}\)-gL could have been caused by PB T cells which recognize peptides of the gH\(_{t}\)-gL complex of other human herpesviruses. Parts of the gH:gL complex are conserved among herpesviruses. However, the gD\(_{t}\)-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\(_{t(His)}\)-gL and gD\(_{t}\) 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-γ 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


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