CD4 down-modulation by ganglioside and phorbol ester inhibits human herpesvirus 7 infection

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Recently, data demonstrating that CD4 is an essential component of the receptor for human herpesvirus 7 (HHV-7) as well as for human immunodeficiency virus have been accumulating. Since gangliosides and phorbol esters are known to induce selective down-modulation of cell surface CD4 expression, it might be expected that treatment with these agents would interfere with HHV-7 infection of CD4+ T cells. The present study, undertaken to verify this possibility, demonstrated that addition of monosialoganglioside-GM1 or 12-O-tetradecanoylphorbol 13-acetate effectively induced disappearance of CD4 from the cell surface and also reduced HHV-7 infectivity, as judged by the CPE on virus-infected cells and studies of indirect immunofluorescence, TCID₅₀ and semi-quantitative PCR of the HHV-7 genome. Taken together with previous studies, the present data strongly suggest that the CD4 molecule is a critical component of the receptor for HHV-7.

Human herpesvirus-7 (HHV-7) is a newly recognized herpesvirus which was originally isolated from the CD4+ T cells of a healthy individual (Frenkel et al., 1990). Lusso et al. (1994) and our group (Furukawa et al., 1994) have recently found that HHV-7 infection results in a marked decline in surface CD4 expression and functional alteration of CD4+ T cells. It is well known that such down-modulation of expression of surface CD4, which is the receptor for human immunodeficiency virus-1 (HIV-1), also occurs in HIV-1-infected cells (Hoxie et al., 1986a; Stevenson et al., 1987; Salmon et al., 1988; Kawamura et al., 1989; Geleziunas et al., 1994). Therefore, it can be anticipated that the binding of HHV-7 to the CD4 molecule is one of the causes of the down-modulation of surface CD4 expression in HHV-7-infected cells. Lusso et al. (1994) have recently reported that anti-CD4 MAb and the soluble form of CD4 inhibit HHV-7 infection. Moreover, they showed that exposure of CD4+ T cells to HHV-7 interfered with infection by HIV-1 and that persistent infection with HIV-1 or treatment with the soluble form of HIV-1 glycoprotein gp120 rendered CD4+ T cells resistant to HHV-7 infection. Similar data have also been obtained in a series of experiments performed by our group, strongly suggesting that the CD4 molecule is the major receptor for HHV-7.

Gangliosides are acidic glycolipids which bind to specific sites on T cells and induce selective loss of CD4 without affecting other surface molecules (Offner et al., 1987; Kawaguchi et al., 1989). Therefore, the treatment of CD4+ T cells with gangliosides is a useful method for examining the role of the CD4 molecule. Phorbol esters, such as 12-O-tetradecanoylphorbol 13-acetate (TPA), are also known to rapidly down-modulate CD4 expression on the cell surface (Hoxie et al., 1986b; Neudorf et al., 1991). On the basis of these previous findings, and to verify the possibility that CD4 is the essential component of the binding receptor for HHV-7, the effects of treatment of CD4+ T cells with a ganglioside and TPA on HHV-7 infectivity were examined in the present study.

The RK strain of HHV-7, kindly provided by Dr Niza Frenkel (NIH, Bethesda, Md, USA), and the U1102 strain of HHV-6, kindly provided by Dr John Nicholas (MRC, London, UK), were grown in cord blood mononuclear cells, which were stimulated by phytohaemagglutinin (PHA) as described previously (Frenkel et al., 1990). The indirect immunofluorescence assays for detection of HHV-7 and HHV-6 were performed using HHV-7- and HHV-6-seropositive human sera, as described previously (Yakushijin et al., 1991). The TCID₅₀ was determined as follows. Virus-infected cells suspended in culture supernatant were frozen and thawed, and then sonicated. PHA-stimulated cord blood lymphocytes (2 × 10⁵) suspended in 100 µl RPMI 1640 medium

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supplemented with 10% FCS (10% FCS RPMI 1640) and interleukin-2 (IL-2) were plated into a flat-bottomed microtitre well, and then 100 µl of 10-fold serially diluted sample was added to each well. Eight wells were prepared for each dilution. The plates were incubated in an atmosphere of 5% CO₂ at 37 °C for 7 days, and the CPE was examined with an inverted microscope. TCID₅₀ was calculated by the method of Reed & Muench (1938).

Semi-quantitative PCR assays for the HHV-7 and HHV-6 genomes were performed as follows. Twenty microlitres of 10-fold serially diluted virus sample, which had been prepared as described above, was added to a pellet of 2 x 10⁶ cells of the human T cell line, Jurkat. DNA was extracted from each sample and amplified for 30 cycles using Taq DNA polymerase (Promega) and the following primers: 5' TATCCCAGCTGTTTTCATAGTAAC 3' and 5' GCCTTGCCTAGCTAGATTITTTTG 3' for amplification of HHV-7 DNA (Berneman et al., 1992); and 5' GTGTTTCCATTGTACTGAAACCGGT 3' and 5' TAAACATCAAAGCGTTGCATACAGT 3' for amplification of HHV-6 DNA (Yalcin et al., 1992). The amplified products were electrophoresed on a 2% agarose gel and stained with ethidium bromide. The expected products of HHV-7 and HHV-6 DNAs that were obtained using these primers are 186 bp and 776 bp long, respectively.

The effect of gangliosides and phorbol esters on HHV-7 infectivity in CD4⁺ T cells was examined as follows. As the addition of FCS to the culture medium inhibits the effect of gangliosides on CD4 expression (Chieco-Bianchi et al., 1989), the human CD4⁺ T cell line Sup-T1, which is susceptible to HHV-7, was washed and suspended in serum-free RPMI 1640 medium at a concentration of 1 x 10⁶ cells/ml. Monosialoganglioside-GM1 (GM1; Advanced Immuno Chemical Incorporated) was then added to 1 ml of cell suspension at various concentrations, and incubated at 37 °C for 1 h. Then the cells were inoculated with 100 µl HHV-7 stock (10⁴⁵ TCID₅₀/ml). After incubation for 1 h at 37 °C the cells were washed to remove the cell-free virus and suspended in 1 ml 10% FCS RPMI 1640 and cultured in an individual well of a 24-well plate. In order to examine the effect of phorbol esters on HHV-7 replication, TPA (Sigma) was added to 1 x 10⁷ Sup-T1 cells suspended in 1 ml 10% FCS RPMI 1640 at various concentrations. After incubation for 4 h at 37 °C, the cells were washed to remove TPA and resuspended in 1 ml 10% FCS RPMI 1640. Then the cells were inoculated with 100 µl of HHV-7 stock and cultured in an individual well of a 24-well plate. In order to examine the effects of GM1 and TPA on HHV-7 replication after viral entry, GM1 or TPA was added to Sup-T1 cells, which had been inoculated with HHV-7 and cultured for 2 h. The effects of GM1 and TPA on HHV-6 replication were examined using the HHV-6-susceptible cell line, JHHAN, as described above. After 6 days for HHV-7 and 5 days for HHV-6, the cultured cells were observed with an inverted microscope to detect CPE, assayed for TCID₅₀ and analysed by indirect immunofluorescence and PCR for detection of the viral antigen and genome, respectively.

We first examined the effects of different concentrations of GM1 and TPA on Sup-T1 cell surface CD4 expression. As shown in Fig. 1, rapid down-modulation of cell surface CD4 following treatment with GM1 or TPA was detected by flow cytometry. The minimum concentration of GM1 and TPA needed for producing marked loss of expression of CD4 was 10 µg/ml and 1 ng/ml, respectively. Mean fluorescence intensities of CD4, plotted on a log-scale, on untreated Sup-T1 cells, Sup-T1 cells treated with GM1 (10 µg/ml) for 1 h, and Sup-T1 cells treated with TPA (1 ng/ml) for 4 h were 92-8, 5-8, and 7-3, respectively. As shown in previous reports, CD4 was re-expressed on the cell surface after overnight culture of GM1-treated Sup-T1 cells in FCS-

**Fig. 1.** Down-modulation of surface CD4 in Sup-T1 cells by GM1 and TPA. Sup-T1 cells were (a) untreated or treated with (b) 10 µg/ml GM1 or (c) 1 ng/ml TPA as described in the text. Cells were stained with FITC-conjugated anti-CD4 MAb, Leu3a, and CD4 surface expression was analysed by flow cytometry. Results from stainings with the control antibody, FITC-conjugated mouse IgG, are shown as thin lines.
containing culture medium. On the other hand, the level of expression of CD4 on the surface of Sup-T1 cells, which had been treated with TPA for 4 h and cultured thereafter for 16 h in TPA-free medium, was still lower than that found in untreated cells (data not shown).

The effects of treatment of Sup-T1 cells with GM1 and
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Table 1. Infectivity titre of HHV-7 in Sup-T1 cells treated with GM1 or TPA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>HHV-7 titre (TCID_{50}/ml)</th>
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<tbody>
<tr>
<td>None</td>
<td>10^{+5}</td>
</tr>
<tr>
<td>GM1 (100 µg/ml)</td>
<td>10^{+4}</td>
</tr>
<tr>
<td>GM1 (10 µg/ml)</td>
<td>10^{+4*}</td>
</tr>
<tr>
<td>TPA (10 ng/ml)</td>
<td>10^{+3}</td>
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<tr>
<td>TPA (1 ng/ml)</td>
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TPA on HHV-7 infection are shown in Fig. 2. As shown in Fig. 2 (a, b), pre-treatment with both GM1 and TPA resulted in inhibition of HHV-7 replication, as judged by the CPE on HHV-7-infected cells and indirect immunofluorescence measurements using HHV-7-seropositive serum. HHV-7-infected Sup-T1 cells showed a CPE after 6 days that was typical of HHV-7 infection, whereas such CPE was hardly detected in GM1-treated and TPA-treated cells that were similarly inoculated with HHV-7. Indirect immunofluorescence assays showed that the HHV-7 antigen was expressed in more than 80% of cells at day 6 after infection. On the other hand, less than 5% of cells were positive for HHV-7 antigen when treated with GM1 or TPA before virus inoculation. The inhibition of HHV-7 replication by GM1 and TPA was also detected by semi-quantitative PCR (Fig. 2 c) and determination of TCID_{50} values (Table 1). These quantitative analyses of virus replication parameters showed that the treatments of Sup-T1 cells with GM1 and TPA in our experimental system resulted in an approximately 1000-fold reduction of HHV-7 replication. Although cell surface CD4 was undetectable after treating Sup-T1 cells with GM1 at a concentration of 10 µg/ml in serum-free medium, an approximately 10-fold greater concentration of GM1 was needed to inhibit HHV-7 replication. This phenomenon might have been caused by the formation of ganglioside–albumin complexes, resulting in the prompt reappearance of CD4 on the cell surface.

On the other hand, treatment of Sup-T1 cells with GM1 or TPA after virus inoculation showed no inhibitory effect on HHV-7 replication (Fig. 2 a–c). In addition, in contrast to the marked inhibitory effect on HHV-7 replication, GM1 and TPA did not affect HHV-6 replication (Fig. 2 d–f).

The present study showed that absence of cell surface CD4 expression resulted in inhibition of HHV-7 infection and confirmed the recent finding that the CD4 molecule is the major component of the HHV-7 receptor. The following findings indicated that the inhibitory effects of GM1 and TPA on HHV-7 replication were due not to their toxicity on cells or virus but rather to CD4 down-modulation. First, addition of GM1 and TPA to the culture medium of CD4+ T cells that had been inoculated with HHV-7 did not affect HHV-7 replication. Second, treatment of CD4+ T cells with GM1 and TPA did not inhibit the infectivity of another CD4+ T-lymphotropic herpesvirus, HHV-6.

The present work and the recent study by Lusso et al. (1994) have shown that HHV-7 and HIV-1 compete for cell surface CD4 as their binding receptor. Identification of the HHV-7 structure that binds to the CD4 molecule might allow the development of a subcomponent peptide derived from HHV-7 that selectively inhibits HIV binding but does not affect CD4+ T cell functions.

References


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