Human herpesvirus 7 infection of CD4+ T cells does not require expression of the OKT4 epitope

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To evaluate the role of the OKT4 epitope in human herpesvirus 7 (HHV-7) infection, we studied the susceptibility to HHV-7 infection of CD4+ T cells isolated from two individuals with OKT4 epitope deficiency. HHV-7-infected OKT4-Leu3a+ T cells exhibited the characteristic cytopathic effect, reactivity with HHV-7-seropositive serum by immunofluorescence and down-modulation of surface CD4 in a manner similar to HHV-7-infected OKT4-Leu3a+ T cells. A semiquantitative PCR revealed that the amounts of HHV-7 replicated in OKT4+Leu3a+ T cells and OKT4-Leu3a+ T cells were not significantly different. Although it has been reported that OKT4 monoclonal antibody efficiently inhibits HHV-7 infection, the present study demonstrated that the interaction of HHV-7 with CD4+ T cells does not require participation of the epitope defined by OKT4 monoclonal antibody.

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). It has recently been proposed that the CD4 molecule is an essential component of the receptor for HHV-7 based on the following evidence. First, HHV-7 infection results in a marked decline of CD4 expression (Frenkel et al., 1994; Furukawa et al., 1990). Second, monoclonal antibodies (MAbs) against CD4 and the soluble form of CD4 inhibit HHV-7 infection of CD4+ T cells (Lusso et al., 1994). Third, exposure of CD4+ T cells to HHV-7 interferes with infection by human immunodeficiency virus type 1 (HIV-1), and persistent infection by HIV-1 or treatment with the soluble form of HIV-1 gp120 renders CD4+ T cells resistant to HHV-7 infection (Lusso et al., 1994). Fourth, treatment of CD4+ T cells with ganglioside or phorbol ester induces CD4 down-modulation and reduces the susceptibility to HHV-7 infection (Yasukawa et al., 1995).

The CD4 molecule consists of a 372 amino acid extracellular segment composed of four tandem immunoglobulin-like domains, V1, V2, V3, V4, a 23 amino acid transmembrane domain, and a 38 amino acid cytoplasmic segment (Maddon et al., 1985). Infection by both HIV-1 and HHV-7 is inhibited by MAbs against the V1 domain of CD4, such as OKT4A and Leu3a (Dalgleish et al., 1984; Lusso et al., 1994). However, HHV-7 infection is also efficiently inhibited by MAb OKT4, which binds to the V3 domain of CD4. The possibility that inhibition of HHV-7 infection by OKT4 might be the result of the disappearance of the whole CD4 molecule from the cell surface by internalization of the CD4–OKT4 complex is unlikely, since HIV-1 infection is not inhibited by OKT4. These data suggest that the OKT4-binding epitope is essential for the attachment or entry of HHV-7. An inherited variant of CD4 has been recognized, in which the OKT4 epitope is not expressed, whereas regions detected by other MAbs against CD4 are expressed normally (Bach et al., 1981; Joffe & Rabson, 1984; Sato et al., 1984). In order to evaluate the role of the OKT4 epitope in HHV-7 infection, we studied the susceptibility to HHV-7 infection of CD4+ T cells from two individuals with OKT4 epitope deficiency. We also looked at whether individuals with OKT4 epitope deficiency were infected with HHV-7 by testing for anti-HHV-7 antibody in their sera.

In order to define the alterations in CD4 molecules with OKT4 epitope deficiency, we examined the DNA sequence encoding OKT4 epitope-deficient CD4. The full-length cDNA for CD4 was amplified with primers 5’ AACAAAGTGGTGCTGGGCAAA 3’ and 5’ AATGGGGCTACATGTTTTCTGCTGGGCAA 3’ and 5’ AATGGGGCTACATGTTTTCTGCTGGGCAA 3’ (Maddon et al., 1985) using RT–PCR on mRNAs extracted from peripheral blood lymphocytes of individuals with OKT4 epitope deficiency and a control individual. The cDNAs for CD4 molecule were sequenced using the dideoxy chain termination technique (Sanger et al., 1977).

The RK strain of HHV-7, kindly provided by Niza Frenkel (NIH, Bethesda, Md., USA), was grown in cord blood mononuclear cells, which were stimulated with phytohaemagglutinin (PHA), as described previously (Frenkel et al., 1990). Peripheral blood mononuclear cells were isolated from two healthy unrelated individuals with OKT4 epitope defi-
ciency and a control individual by Ficoll-Conray gradient centrifugation. CD4+ T cells were enriched by treatment of the peripheral blood mononuclear cells with MAbs against CD8, CD56 and complement, as described previously (Yasukawa et al., 1989). CD4+ T cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), PHA and interleukin-2 for 5 days. PHA-stimulated CD4+ T cells were then washed and suspended in RPMI 1640 medium supplemented with 10% FCS, and inoculated with HHV-7 at the approximate m.o.i. of 0-1 TCID50. The cells were cultured in a 5% CO2 incubator at 37 °C until cytopathic effects (CPE) became detectable.

The titre of the antibody against HHV-7 was determined by indirect immunofluorescence assay as described previously (Yakushijin et al., 1991). Briefly, HHV-7-infected cord blood lymphocytes were mounted on glass slides and fixed in cold acetone. Serially diluted serum samples were applied to the slide and incubated for 30 min at 37 °C. After washing, fluorescein isothiocyanate (FITC)-conjugated goat anti-human IgG (Cappel, West Chester, Pa., USA) was added and incubated for 30 min at 37 °C. After washing, the slides were examined using a fluorescence microscope. The reciprocal titre of serum dilution was expressed as the antibody titre.

Replication of HHV-7 in the CD4+ T cells was determined by the detection of typical CPE and by indirect immunofluorescence assays using HHV-7-seropositive human serum, as described above. A semiquantitative PCR for the HHV-7 genome was performed as follows. HHV-7-infected cells (1 x 10⁶/ml) were frozen, thawed and sonicated. Twenty µl of 10-fold serially diluted virus sample was added to a pellet of 2 x 10⁷ Jurkat cells (human T cell line). DNA was extracted from each sample and amplified for 30 cycles using Taq DNA polymerase (Promega) and the primers 5' TATCCCAGCTGGTTTTCATATAGTAAC 3' and 5' GCCTTGCGGTAGCACATTTTTTG 3' (Berneman et al., 1992). The amplified products were electrophoresed on a 2% agarose gel and stained with ethidium bromide. The expected product of HHV-7 DNA obtained using these primers consists of 186 bp.

Expression of surface CD4 on HHV-7-infected and uninfected cells was analysed by direct immunofluorescence using a flow cytometer. Cells were stained with FITC-conjugated MAbs OKT4 (Ortho Diagnostic Systems) or Leu3a (Becton-Dickinson). Cells to be used as unstained negative controls were incubated with FITC-conjugated mouse monoclonal IgG (Becton-Dickinson).

Sequencing of cDNA encoding OKT4-epitope deficient CD4 revealed a single nucleotide base change (CGG to TGG) resulting in substitution of tryptophan at position 240 for arginine in the V3 domain of CD4 (Fig. 1). This change results in an alteration of hydrophobicity and may cause a conformational change in the V3 domain of CD4.

It appeared that both OKT4+ and OKT4- CD4+ T cells became larger after inoculation with HHV-7, showing the typical CPE of HHV-7. We and other investigators have
reported previously that HHV-7 infection induces a marked decrease in surface CD4 expression (Lusso et al., 1994; Furukawa et al., 1994). Therefore, we first examined whether down-regulation of surface CD4 was also induced in OKT4-CD4-T cells by HHV-7 infection, as HHV-7-infected OKT4+CD4+ T cells. (data not shown). These data suggest that HHV-7 can infect OKT4-CD4+ T cells as well as OKT4+CD4+ T cells.

To confirm the productive infection of HHV-7 in OKT4-CD4+ T cells, we next performed indirect immunofluorescence using HHV-7-seropositive serum. A productive infection of OKT4-CD4+ T cells by HHV-7 was documented 6 days after virus inoculation, by detection of bright immunofluorescence (data not shown). Replication of HHV-7 in OKT4-CD4+ T cells was also demonstrated by semi-quantitative PCR. Fig. 3 shows that approximately the same amounts of HHV-7 genome were detected in OKT4+ and OKT4-CD4+ T cells 6 days after virus inoculation. These data indicate that infection of CD4+ T cells by HHV-7 does not require expression of the OKT4 epitope.

Finally, we examined whether two individuals with OKT4 epitope deficiency were infected with HHV-7 by testing for anti-HHV-7 antibody in their sera. They were both found to be seropositive for HHV-7. Their serum titres of anti-HHV-7 antibody, determined by indirect immunofluorescence, were 80 in both cases.

It has been shown recently that CD4 is an important component of the receptor for HHV-7. Lusso et al. (1994) have reported that MAb OKT4, which binds the V3 domain of CD4, as well as MAbs against the V1 domain of CD4, inhibit HHV-7 infection. Similar data have also been obtained in a series of experiments performed by our group. The present study was performed to clarify the role of the OKT4 binding epitope on HHV-7 infection using CD4+ T cells with OKT4 epitope deficiency. The results showed that HHV-7 infection of CD4+ T cells does not require participation of the epitope defined by MAb OKT4, as is also the case for HIV-1 infection (Hoxie et al., 1986). This conclusion was confirmed by the detection of anti-HHV-7 antibody in the sera of two individuals with OKT4 epitope deficiency.

Recent studies on Japanese families with OKT4 epitope deficiency have demonstrated a single nucleotide substitution (CGG to TGG), resulting in arginine being replaced by tryptophan at position 240 in the V3 domain of CD4 (Takenaka et al., 1993). DNA sequencing of the present cases revealed the identical nucleotide substitution to that reported previously. This amino acid substitution results in a change in hydrophobicity at positions 239 and 240 from that of the CD4 molecule bearing the OKT4 epitope, giving rise to a conformational change in CD4 which accounts for the lack of reactivity with MAb OKT4.

Although the precise mapping of the HHV-7-binding regions on CD4 is unknown, the finding that treatment of CD4+ T cells with MAbs against the V1 domain of CD4 and the soluble form of HIV-1 gp120 renders them resistant to HHV-7 infection strongly suggests that the V1 domain of CD4 is a critical component of the receptor for HHV-7 as well as for HIV-1. On the other hand, an MAb against the V3 domain of CD4 inhibited HHV-7 infection but not HIV-1 infection. The mechanism responsible for the inhibition of HHV-7 infection by MAb OKT4 remains obscure. However, one possibility is that binding of OKT4 with the CD4 molecule may induce conformational change in a region other than the OKT4-binding site, which is essential for HHV-7 attachment and entry. Further experiments used mutated recombinant CD4 molecules may elucidate the precise structure of the HHV-7-binding regions on CD4.

References


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