Infection of γ/δ T Lymphocytes by Human Herpesvirus 6: Transcriptional Induction of CD4 and Susceptibility to HIV Infection

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Summary

Human herpesvirus 6 (HHV-6), a T-lymphotropic human herpesvirus, is a potentially immunosuppressive agent that has been suggested to play a role as a cofactor in the natural history of human immunodeficiency virus (HIV) infection. We studied the interactions between HHV-6 and γ/δ T lymphocytes, a subset of T cells involved in the protective immune response against specific microorganisms. Polyclonal γ/δ T cell populations, purified from the peripheral blood of healthy adults and activated in vitro with phytohemagglutinin, were exposed to HHV-6, strain GS (subgroup A), at the approximate multiplicity of infection (MOI) of 1. Signs of virus replication were detected as early as 72 h after infection, as documented by immunofluorescence, electron microscopy, and transmission of extracellular virus. Progression of the infection was associated with the appearance of typical cytomorphological changes and, eventually, massive cell death. In contrast, no signs of infection or cytopathic effects were detected after exposure of γ/δ T lymphocytes to HHV-7, a CD4$^+$ T-lymphotropic virus closely related to HHV-6. Polyclonal γ/δ T cells displayed cytolytic activity against both autologous and heterologous target cells infected with HHV-6 and maintained this activity for at least 72 h after infection with HHV-6, despite the high MOI used. As previously documented in mature CD8$^+$ α/β T cells and natural killer cells, HHV-6 infection induced γ/δ T lymphocytes to express de novo CD4 messenger RNA and protein, as detected by reverse transcriptase-polymerase chain reaction and fluorocytometry, respectively. Whereas purified CD4$^-$ γ/δ T cell populations were per se refractory to HIV infection, they became susceptible to productive infection by HIV-1, strain IIIB, after induction of CD4 expression by HHV-6. These results demonstrate that γ/δ T cells can be directly targeted and killed by a herpesvirus and may have implications for the potential role of HHV-6 in AIDS.

After the identification of the TCR, the main antigen-recognition structure on the surface membrane of T lymphocytes, two distinct lineages of T cells have been defined, i.e., α/β and γ/δ T cells, based on the selective expression of different TCR heterodimers associated with CD3 (1). The α, β, γ, and δ genes have a similar genomic organization, despite a more limited combinatorial potential for γ and δ, and undergo a similar somatic rearrangement during the process of T cell differentiation, albeit differently timed (1). Our knowledge of the physiology and function of γ/δ T cells is still limited, but it is clear that they respond to antigens in a different fashion compared to α/β T cells. Whereas α/β T cells recognize peptide antigens combined with either class I or II MHC molecules, the response of γ/δ T cells is apparently not restricted by polymorphic MHC elements (1). Moreover, unlike α/β T cells, γ/δ T lymphocytes do not usually express, along with the CD3/TCR complex, the CD4 or CD8 antigens, two membrane glycoproteins with an accessory function in the process of antigen-mediated T cell activation (1).

The role of γ/δ T cells in the protective immune response against invading microorganisms has only recently started to be unraveled. Recent in vivo data indicate that γ/δ T cells play a unique defensive role against Listeria monocytogenes in mice, acting in synergy with α/β T cells (2, 3). In vitro, γ/δ T cells proliferate, secrete cytokines, and display cytotoxic activity in response to different antigens, including viruses (e.g., Herpesviridae), bacteria (e.g., Mycobacteria), and protozoa (e.g., Plasmodia) (1). It is interesting, however, that γ/δ T cells can also be activated in vitro by host-encoded antigens, such as heat-shock proteins (1). Thus, the in vivo response of γ/δ T cells to microorganisms may be, at least in part, secondary to the effects that the infection induces in the host, suggesting a possible role of γ/δ T cells in the regulation of the immune response and/or in self-reactive immune phenomena.

Human herpesvirus 6 (HHV-6)[1] (4) was the first T-lymphotropic human herpesvirus (5) to be discovered. Despite its primary tropism for CD4^{+} T cells, HHV-6 is in fact an "immunotropic" herpesvirus, because it can also infect CD8^{+} T cells (6), NK cells (7), and in vitro immortalized B cells (8), as well as, albeit nonproductively, cells of the mononuclear phagocytic system (9, 10). In all these cell types, HHV-6 induces dramatic cytopathic changes, suggesting that it may act as an immunosuppressive agent in vivo. Moreover, a series of positive viral interactions has been documented between HHV-6 and HIV, the causative agent of AIDS, leading to the hypothesis that HHV-6 may accelerate the natural course of HIV infection in coinfected patients (11). This concept has been corroborated by the recent demonstration that HHV-6 infection is active and widespread in terminal AIDS patients (12, 13). Diverse anomalies of both the cellular and humoral arms of the immune system have been reported in patients with AIDS, including γ/δ T cell alterations (14–16). In this study, we have investigated the susceptibility of γ/δ T cells to infection by HHV-6 and the interactions between HHV-6 and HIV in γ/δ T cells.

Materials and Methods

Enriched γ/δ T Cell Populations. PBMC were obtained from normal adult volunteers by separation over a Ficoll-Hypaque gradient (Pharmacia, Piscataway, NJ). To enrich γ/δ T cells, a multiple rosetting procedure with goat anti–mouse IgG–coated magnetic microspheres (Dynal, Oslo, Norway) was used, as previously described (5). Briefly, the cells were washed twice with PBS and incubated for 30 min at 4°C with a cocktail of mAbs directed toward CD4 (Leu3a), CD8 (Leu2a), CD14 (LeuM3), CD16 (Leu11b), CD19 (Leu12), CD20 (Leu16), CD56 (Leu19) (Becton Dickinson & Co., Mountain View, CA) and TCR-α/β (T Cell Diagnostics, Cambridge, MA). All the mAbs were used at a final concentration of 5 μg/ml. The cells were then washed repeatedly with cold PBS and incubated under continuous rotation at 4°C with the immunomagnetic beads at a 40:1 ratio between beads and putatively positive cells. After 30 min, the beads were removed with a magnet and the cells remaining in suspension transferred to a new vial. The latter operation was performed again to remove all the residual beads. The cells were subsequently pelleted and incubated a second time for 30 min with immunomagnetic beads at a ratio of 40:1 between beads and 5% of the original number of putatively positive cells. After removing the beads twice with a magnet, the cells were washed and cultured in RPMI medium supplemented with 10% fetal bovine serum and 1 μg/ml of purified PHA (Wellcome, Burgwedel, Germany). 24 h later, partially purified human IL-2 (Boehringer Mannheim, Mannheim, Germany) was added to the cultures at a concentration of 10 U/ml. The enriched cell populations were tested for purity by fluorocytometry with the mAbs used for selection, as well as with mAb TCR-δ1 (T Cell Diagnostics). If purity was not superior to 98%, the cells were subjected to two additional cycles of purification 72 h after PHA stimulation and then retested for purity. If purity was again unsatisfactory, two additional purification cycles were performed 96 h after PHA stimulation. At this time, fluorocytometric analysis con-

sistently revealed <1.0% cells positive for any of the mAbs used for negative selection and >98% of the cells exhibiting the TCR δ1 marker.

Viruses, Infection Procedure, and Monitoring. The HHV-6 stock, strain GS (4), was prepared from infected cultures of human umbilical cord blood mononuclear cells, as previously described (5). For infection, pelleted cells were incubated with the stock virus at the approximate multiplicity of infection (MOI) of 1, i.e., one infectious dose per cell. After 1 h at 37°C, the cells were washed with prewarmed culture medium and resuspended in complete medium supplemented with 1 U/ml of IL-2.

The HIV-1 stock, strain IIIB, was grown in the continuous cell line H9 (17). The cells were infected with 10⁵ cpm of reverse transcriptase (RT)/10⁶ cells for 1 h at 37°C. To minimize the amount of residual virus from the inoculum, the cells were washed three times in a 15-ml volume of culture medium. All the residual medium was carefully removed each time after pelleting. The infection was monitored by measuring the amount of HIV-1 p24 Gag protein in the culture supernatants using a commercial antigen capture ELISA test (Dupont NEN, Boston, MA). mAb OKT4a (Ortho Diagnostics, Raritan, NJ), previously dialyzed and sterilized by filtration through a 0.2 μm-membrane, was used to pretreat the cells at 5 μg/ml and was maintained in the cultures at the same concentration thereafter.

mAbs and Immunologic Analyses. The mAbs used for fluorocytometric analysis included Leu4 and Leu3a conjugated with PE (Becton Dickinson & Co.), FITC-conjugated TCR-δ1 and TCR-α/β, purified γV2 (a) (anti-Vγ9) and βF1 (T Cell Diagnostics), and 13D6, directed toward an HHV-6 envelope protein of 120 kD (a gift of Dr. Bala Chandran, University of Kansas, Kansas City, KS) (18). FITC-conjugated goat antiserum to murine IgG (Sigma Chemical Co., St. Louis, MO) was used as a second-layer reagent. Because the βF1 mAb recognizes an epitope that is not exposed on live cells, labeling with this mAb was performed on fixed cells. For fixation, the cell pellet was incubated with 2% paraformaldehyde for 10 min at room temperature, washed twice with PBS, and then treated with 70% ethanol at −20°C for 5 min. As a positive control, PHA-activated CD4^{+} T cells from adult peripheral blood were used: >98% were stained by βF1 under these conditions. Fluorocytometric analysis was performed on a FACScan® analyzer (Becton Dickinson & Co.). In infected cultures, dead cells were removed before immunofluorescence (IF) analysis by centrifugation on a Ficoll gradient. In addition, appropriate gating was used to eliminate from the analysis residual dead cells and cellular debris. Controls were stained with mouse IgG1 and IgG2 irrelevant antibodies conjugated with either fluorochromes. At least 10,000 events were accumulated in each experiment.

To evaluate the level of HHV-6 or HHV-7 infection, indirect IF analysis was performed on acetone-fixed cells, as described (5), with mAb 9A5D12 (a gift of Dr. Balachandran) (18), directed toward an HHV-6 nuclear phosphoprotein with a molecular mass of 41/110 kD, but cross-reacting with an analogous protein of HHV-7 (19).

⁵¹Chromium-Release Assay for Cytotoxicity. For the cytotoxicity studies, a standard 4-h ⁵¹Cr-release test was employed, as described in detail elsewhere (20). Before labeling, HHV-6–infected target cells were centrifuged over a Ficoll gradient to remove dead cells. After this procedure, the viability was >80%. The percent specific lysis was calculated according to the following formula: 100 × [(test release − spontaneous release)/(maximal release − spontaneous release)].

RT-PCR for CD4 Messenger RNA (mRNA). For mRNA analysis, infected γ/δ T cells were harvested at day 8 after exposure

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[1] *Abbreviations used in this paper:* HHV-6, human herpesvirus type 6; IF, immunofluorescence; MOI, multiplicity of infection; mRNA, messenger RNA; RT, reverse transcriptase.

to HHV-6, when >50% expressed virus-specific antigens, as assayed by indirect IF. Uninfected homologous cells were cultured in parallel and harvested at the same time. Additional controls included the CD4-negative B cell line Raji, as well as normal PBMC previously activated with PHA in vitro. The RT-PCR assay for CD4 was performed on total cellular RNA as described (7). The primers used for amplification were: CD4.1, 5'-GCAGTGGCG-AGCTGTGGT-3'; CD4.2, 5'-GGGTCCCCACACCTCACAGG-3'; the probe (CD4.3) was 5'-GAACCTGGTGGTGATGAGAGC-CACTCAGCT-3'.

Results

Productive and Cytopathic Infection of γ/δ T cells by HHV-6_GS. Enriched populations of γ/δ T lymphocytes were derived from the peripheral blood of healthy adult individuals by repeated negative immunomagnetic selection cycles and activated in vitro with PHA. The residual contamination of α/β T lymphocytes was <1%, as assayed by fluorocytometry. Activated γ/δ T cells were exposed to HHV-6, strain GS (the prototype of the A subgroup of HHV-6) (4), at an approximate MOI of 1 (i.e., one infectious dose per cell). The infection was monitored by indirect IF, electron microscopy, and transmission of released, extracellular virus to PHA-activated cord blood mononuclear cells. Signs of productive infection started to be detectable at day 3 after exposure to the virus, as demonstrated by the expression of a nuclear early-late viral antigen recognized by mAb 9A5D12 (Fig. 1), which is not detectable in cells blocked at the immediate-early/early stage of HHV-6 expression (21). The various steps of HHV-6 morphogenesis (i.e., nuclear assembly of viral nucleocapsids [A], cytoplasmic migration of tegument-coated particles [B],

and extracellular release of mature, enveloped virions [C]) were documented by transmission electron microscopy (Fig. 2). The productive nature of the infection was further confirmed by transmission of cell-free infectious supernatants to cultures of susceptible cells (data not shown).

Starting 4–7 d after infection, a growing proportion of γ/δ T cells exhibited the typical HHV-6–induced cytomorphological changes, consisting of size enlargement, refractile appearance, and loss of blastic shape with the acquisition of an evenly rounded cellular profile. Eventually, the viral cytopathic effect induced widespread cell destruction, resulting in the extinction of the cultures at day 10–12 after infection.

Lysis of Autologous and Heterologous HHV-6–infected Cells by Activated γ/δ T Lymphocytes: Late Functional Impairment of γ/δ T Lymphocytes after HHV-6 Infection. Enriched γ/δ T cell populations, previously activated in vitro with PHA and cultured in the presence of IL-2, were assayed for their ability to lyse autologous or heterologous PBMC productively infected by HHV-6_GS in vitro. A high level of specific lysis was observed against autologous infected target cells (Fig. 3 A). Similar levels of activity were detected against heterologous target cells infected with HHV-6 (data not shown), whereas a lower, but still significant activity was seen against the K562 cell line, a classical NK target (Fig. 3 B). In contrast, uninfected autologous PBMC were not lysed (<3% specific lysis at a 30:1 E/T ratio).

To investigate the effects of HHV-6 infection on the lytic ability of γ/δ T cells, the effector cells were themselves infected with HHV-6_GS at an approximate MOI of 1 and subsequently tested, at various days after infection, against autologous HHV-6–infected targets and against K562. As

Figure 1. Expression of viral antigens in γ/δ T lymphocytes infected with HHV-6, strain GS, as assessed by indirect IF with mAb 9A5D12.
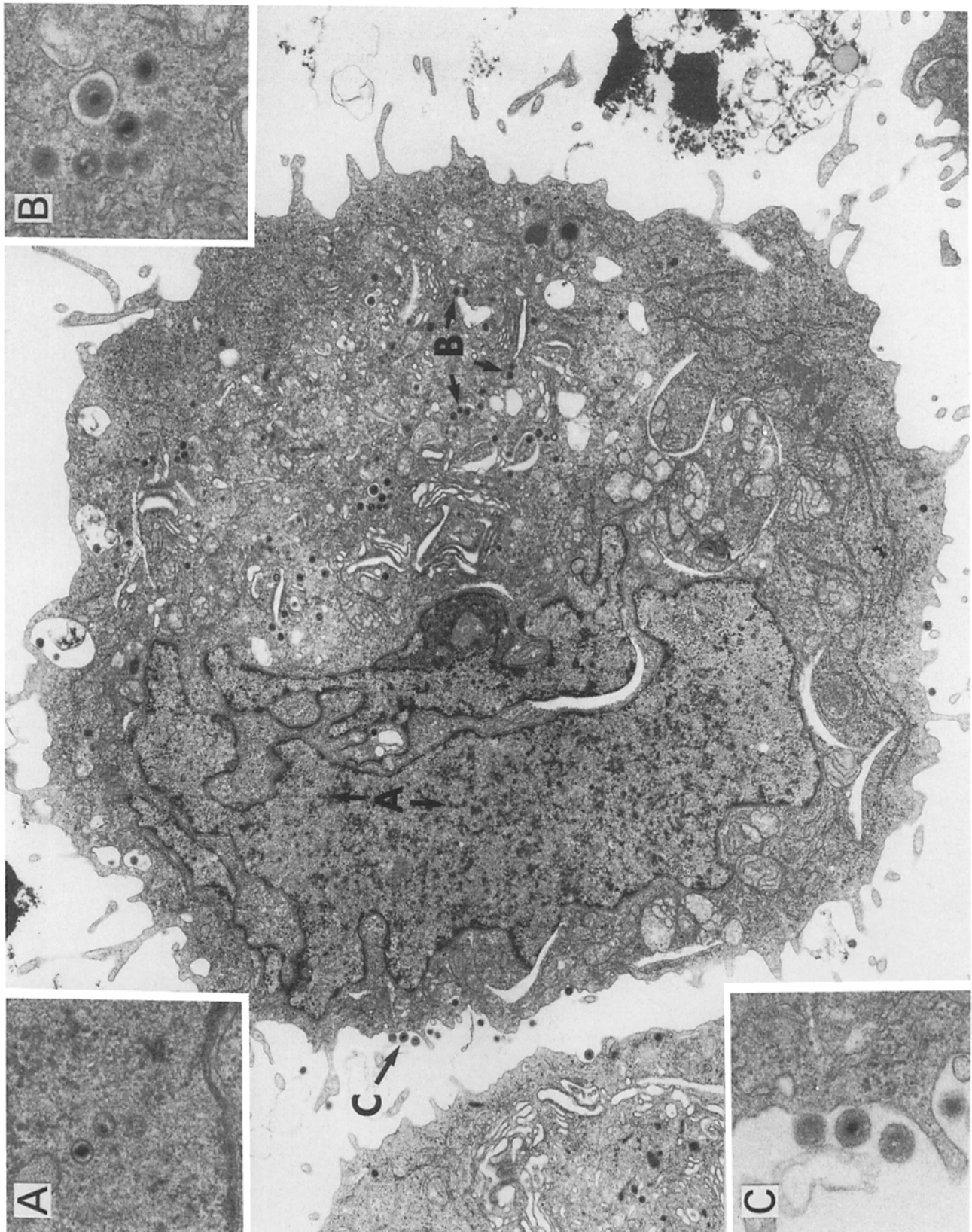
Figure 2. Electron micrograph illustrating different phases of HHV-6 maturation in an infected γ/δ T lymphocyte. Viral nucleocapsids can be seen within the nucleus (A), tegument-coated immature virions inside the cytoplasm (B), and mature virions in the extracellular space (C).
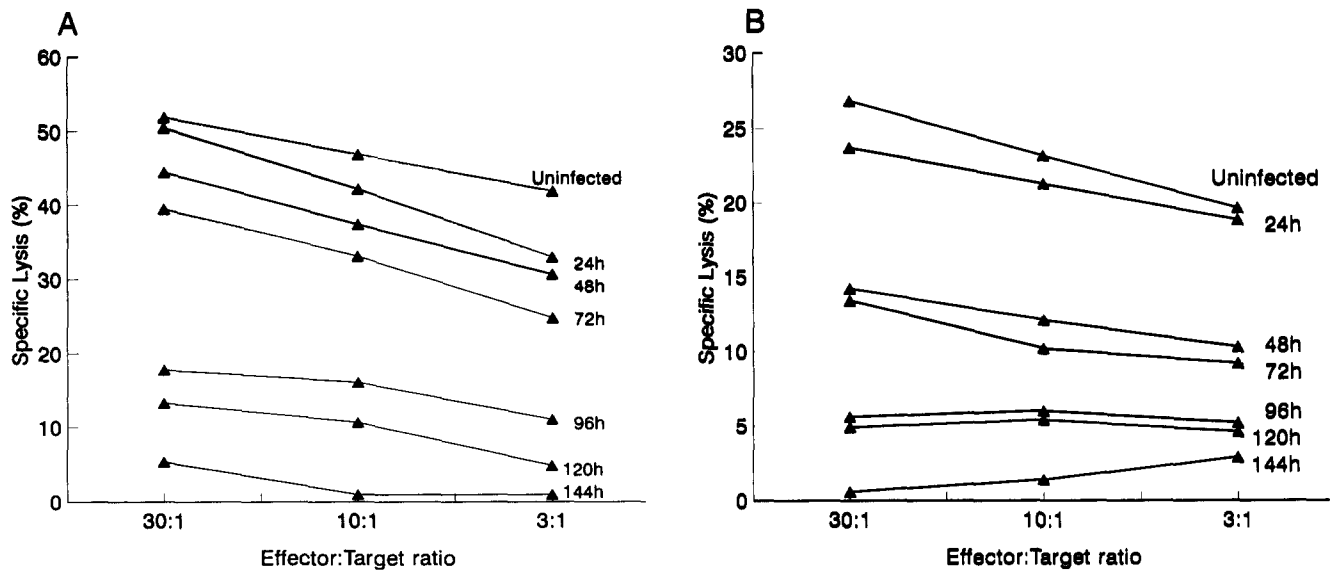
A

60

50

40

Specific Lysis (%)

30

20

10

0

Uninfected

24h
48h

72h

96h

120h
144h

30:1   10:1   3:1

Effector:Target ratio

B

30

25

20

Specific Lysis (%)

15

10

5

0

Uninfected

24h

48h
72h

96h
120h

144h

30:1   10:1   3:1

Effector:Target ratio

Figure 3. Lysis of autologous HHV-6–infected PBMC (*A*) and uninfected K562 (*B*) by γ/δ T cell populations before and at various times after infection with HHV-6. A standard ^{51}Cr-release assay was used.

illustrated in Fig. 3 *A*, the killing ability of γ/δ T cells against infected targets was only minimally affected during the first 72 h after infection, despite the high MOI used. In contrast, the ability to kill K562 was already reduced by ~50% at day 2–3 after infection. However, a more dramatic loss of cytotoxic activity was observed, against both target cell types, at day 4 after infection. At this time, an increasing proportion of effector cells started to express structural viral antigens, as detected by indirect IF. These data demonstrate that γ/δ T cells are functionally impaired by HHV-6 infection, but are not immediately disarmed upon contact with the virus. In particular, the cytotoxic activity against infected targets is lost only at a relatively late stage in the course of the infection, when active virus replication is already taking place.

Fluorocytometric Analysis of HHV-6–infected γ/δ T Cells: De Novo Expression of CD4. The expression of selected surface membrane antigens was analyzed in γ/δ T cells in the course of HHV-6 infection. As illustrated in Fig. 4, >99% of the enriched γ/δ T cells at day 0 expressed CD3 and the γ/δ TCR heterodimer (mAb TCR-δ1), whereas the α/β TCR heterodimer and, in particular, the TCR β chain (mAb βF1) were expressed by <1% of the cells. As expected in peripheral blood γ/δ T lymphocytes, the majority of the cells expressed Vγ9. Less than 1% of the cells expressed CD4, an antigen that is rarely detected in γ/δ T lymphocytes (1) and that was included in the negative selection procedure for the purification of γ/δ T cells.

To elucidate the effects of HHV-6 on γ/δ T cells, fluorocytometric analysis was performed at various days after infection. The level of infection was evaluated by mAb 13D6 which recognizes an envelope-associated HHV-6 antigen present on mature virions (18). In parallel with the progressive appearance of membrane viral antigens, de novo expression of CD4 was dramatically induced in γ/δ T cells in the course of HHV-6 infection (Fig. 4). At day 9 after infection, the CD4 antigen was expressed by >70% of the cells. The induction

of CD4 was a progressive phenomenon already detectable, albeit at very low levels, 3 d after infection. Similar patterns and kinetics of CD4 induction after HHV-6 infection were previously documented in CD8$^+$ α/β T cells (21) and NK cells (7). With the exception of a moderate loss of fluorescence intensity for CD3 and the γ/δ heterodimer, no substantial alterations were observed in the expression of the other antigens tested, in the course of HHV-6 infection. In particular, the proportion of Vγ9$^+$ cells remained virtually constant and the TCR β chain was consistently negative throughout the infection period, suggesting that cell subsets with unusual phenotypes, e.g., β/δ T cells, were not selectively expanded in culture.

To investigate whether the induction of CD4 in HHV-6–infected γ/δ T cells occurred at the transcriptional level, we assayed the expression of CD4 mRNA at day 8 after infection using a RT-PCR system. Uninfected homologous cells were used in parallel as a control. Further controls included PHA-activated human PBMC (>60% CD4$^+$) and the Raji B-lymphoblastoid cell line (CD4$^-$). As illustrated in Fig. 5, no CD4 mRNA was detected, despite the high sensitivity of the PCR test, in uninfected γ/δ T cells (lane 4). However, HHV-6 infection induced de novo expression of CD4 mRNA in γ/δ T cells, as documented by specific hybridization of the CD4 oligonucleotide probe to an amplified cDNA product exhibiting the expected size (299 bp) (lane 5). This result indicates that the induction of CD4 expression in HHV-6–infected γ/δ T cells occurs at the transcriptional level.

Induction of Susceptibility to HIV-1 in γ/δ T Cells by HHV-6 Infection. To elucidate whether the newly induced CD4 could serve as a functional receptor for HIV-1 (22, 23), γ/δ T cells were initially infected with HHV-6 at an approximate MOI of 1 and, 48 h later, exposed to HIV-1, strain IIIB (17). The level of HIV-1 replication was evaluated by measuring the amount of p24 core antigen released into the culture super-
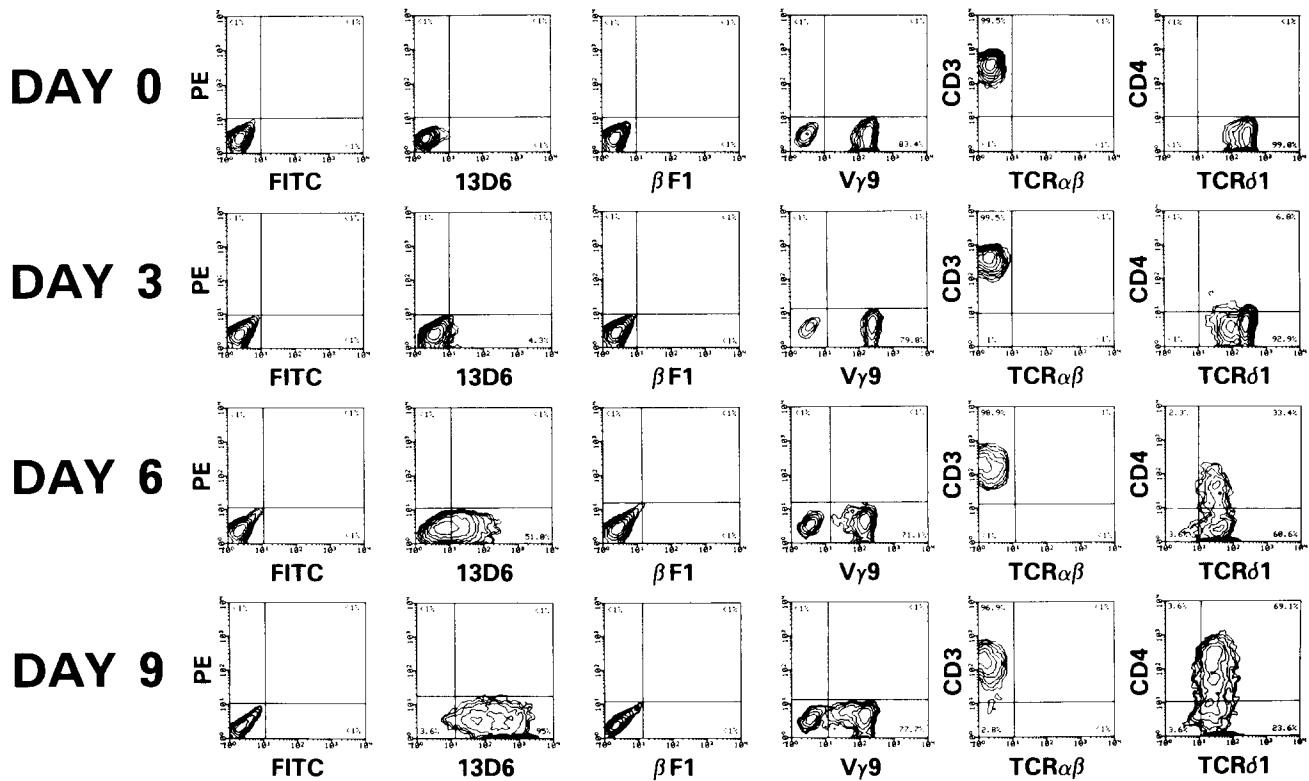
Figure 4. Fluorocytometric analysis of γ/δ T cells in the course of HHV-6 infection (days 0, 3, 6, 9 after infection). FITC and PE denote irrelevant mAbs labeled with either fluorochrome and used as controls.

natants at various days after infection. Consistent with the lack of the CD4 receptor, γ/δ T cells were per se refractory to HIV-1 infection (Table 1). In contrast, after HHV-6 infection, they became susceptible to productive infection by HIV-1, as demonstrated by the growing kinetics of extracellular p24 antigen release. Treatment of the HHV-6–infected cells with an HIV-blocking mAb to CD4 (OKT4a) completely abrogated the release of HIV-1 p24 (Table 1), conclusively demonstrating that the HHV-6–induced CD4 was essential for infection of γ/δ T cells by HIV-1.

Discussion

Although the role of γ/δ T cells in the strategic organization of the immune system has only recently started to be

Figure 5. Expression of CD4 mRNA in γ/δ T cells before and after infection with HHV-6 as determined by RT-PCR on total RNA. Lane *1*: PHA-activated adult PBMC (>60% CD4⁺); lane *2*: PCR reagents (negative control); lane *3*: Raji (CD4⁻ B cell line); lane *4*: uninfected γ/δ T-lymphocyte population; and lane *5*: HHV-6–infected γ/δ T-lymphocyte population at day 8 after infection.

unraveled, increasing evidence indicates that these cells represent an essential component of the protective response against specific microorganisms (1–3). In this study, we documented that HHV-6, a T-lymphotropic herpesvirus, can productively infect γ/δ T lymphocytes, inducing important phenotypic and cytopathic changes. Moreover, we found that γ/δ T cells can recognize and kill HHV-6–infected target cells, suggesting that γ/δ T cells may play a protective role against infection by HHV-6 in vivo. Thus, by directly attacking γ/δ T cells,
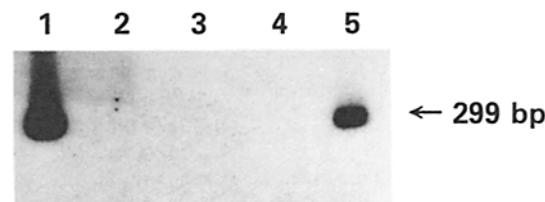
Table 1. *CD4-dependent HIV-1 Infection of γ/δ T Cells after Induction of CD4 by HHV-6*

Viruses used for infection	HIV-1 replication (p24)			
	48 h	72 h	96 h	120 h
	pg/ml			
HIV-1	<1	<1	<1	<1
HHV-6 + HIV-1	22	40	56	171
HHV-6 + HIV-1 + OKT4a	<1	<1	<1	<1

Infection with HIV-1, strain IIIB, was performed 48 h after infection with HHV-6, strain GS, at a time when de novo CD4 expression was already detectable. The anti-CD4 mAb (OKT4a), previously dialyzed, was used at 5 μg/ml and maintained at the same concentration for the entire experiment. Results of a representative experiment are shown.

HHV-6 may seek to escape the immune control of the host and thereby establish persistent infection. Previous studies have elucidated that HHV-6 is an immunotropic herpesvirus that can infect and kill several critical elements of the immune system (4–10). These biological properties suggest that infection with HHV-6 may have broad immunosuppressive effects, although direct in vivo evidence for such action has not yet been provided. Nevertheless, accumulating clinical evidence indicates that HHV-6 may be associated with severe pathology, such as pneumonitis, encephalitis, and bone marrow suppression, in patients subjected to immunosuppressive regimens after bone marrow or organ transplantation (24–26) and in patients with AIDS (13). In HIV-infected patients, however, HHV-6 has been suggested to play a more substantial pathogenetic role (11). Diverse clinical and experimental observations indicate that HHV-6 may contribute, directly or indirectly, to the destruction of CD4$^+$ T cells, a pathological hallmark of AIDS, and thereby expedite the course of the disease. In vitro, HHV-6 can coinfect individual CD4$^+$ T cells with HIV, accelerating the kinetics of HIV expression and cell death (27). This synergistic effect may be due to multiple mechanisms, including the transactivation of the regulatory sequences contained in the long terminal repeat of HIV (27–29), the release of HIV-activating cytokines (30) and the expansion of the range of cells susceptible to HIV by inducing de novo expression of the CD4 receptor (7, 21). In this study, we found that γ/δ T cells, after HHV-6 infection, are induced to express CD4 on their surface membrane, thus becoming susceptible to productive infection by HIV-1. Given the potential role of HHV-6 in AIDS, it is tempting to speculate that this virus might contribute, directly or indirectly, to the γ/δ T cell abnormalities that have been described in patients infected with HIV (14–16). Damage to γ/δ T lymphocytes could predispose patients to infection by specific microorganisms that these cells help to control, including some (e.g., *Mycobacteria*) that are an important cause of morbidity and mortality in AIDS (31).

We found that polyclonal γ/δ T cells induce lysis of both autologous and heterologous target cells infected by HHV-6, as previously demonstrated for NK cells (20). Unlike γ/δ T cells, however, polyclonal NK cells were not productively infected in vitro by HHV-6, despite rapid internalization of the virions (7). Because polyclonal NK cells efficiently recognize and kill HHV-6–infected targets, we hypothesized that active recognition of the target might induce resistance to HHV-6 infection in the NK effectors (7). Consistent with this concept, productive HHV-6 infection was documented in NK clones lacking the ability to kill HHV-6–infected targets, but not in clones with a high lytic activity against infected cells. In the present study, uncloned γ/δ T cells were readily infected by HHV-6, in spite of their ability to kill infected targets. Whether γ/δ T and NK cells receive different activatory signals from infected targets or display a different repertoire of reactive events (e.g., cytokine secretion, membrane alterations) remains to be established. We observed that γ/δ T cells maintained their cytolytic activity against infected cells for several days after exposure to HHV-6. The subsequent loss of cytotoxic ability coincided with the early stages of extracellular virus production (day 4 after infection), at a time when the cellular DNA and protein synthesis machineries start to be overtaken by the herpesvirus. The lytic effect against K562 started to decrease even at an earlier time after infection (day 2), suggesting that the functional impairment induced by HHV-6 in γ/δ T cells is generalized and not specific for the recognition and/or killing of virus-infected cells. In another herpesvirus system, HSV-1, a rapid loss of killing ability has been documented after exposure of CTL, NK, or LAK cells to infected targets (32–35). Direct infection by HSV-1 has been proposed as a possible mechanism for such functional impairment in CTL and LAK cells (34, 35). However, when cell-free HSV-1 was used, the killing ability of CTLs was lost only at exceedingly high MOI levels. Cell-free virus inocula were also used in our experiments with HHV-6, but not at similar MOI levels. However, the fact that uninfected γ/δ T (this report) and NK cells (20) display a significant level of cytotoxic activity in vitro against HHV-6–infected targets suggests that HHV-6, at variance with HSV-1, is not able to promptly disarm cytolytic effectors. Further studies are needed to elucidate whether herpesviruses exploit common mechanisms for impairing the function of antiviral effector cells and thereby elude the immune surveillance of the host.

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