Human Herpesvirus 7 Infection of Lymphoid and Myeloid Cell Lines Transduced with an Adenovirus Vector Containing the CD4 Gene

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It has been reported recently that CD4 is a major component of the receptor for human herpesvirus 7 (HHV-7), which has been newly identified as a T-lymphotropic virus. To investigate further the role of CD4 in HHV-7 infection, we examined the susceptibility to HHV-7 infection of various CD4-negative or weakly positive cell lines into which the cDNA for CD4 was transferred using an adenovirus vector (Adex1CACD4). Of 13 cell lines transduced with Adex1CACD4, including T-lymphoid, B-lymphoid, monocytoid, and myeloid cell lines, one T-lymphoid cell line, one monocytoid cell line, and two cell lines established from the blast crisis of chronic myelogenous leukemia showed high susceptibility to HHV-7 infection. Taken together with the results of previous studies, these data suggest strongly that CD4 is a major component of the binding receptor for HHV-7. This study also shows that HHV-7 may be able to infect CD4-positive hematopoietic precursor cells as well as T lymphocytes.

Human herpesvirus 7 (HHV-7) is a newly identified T-lymphotropic virus that was originally isolated from peripheral blood $CD4^+$ T lymphocytes (11). It has been found that HHV-7 prevalently infects individuals at young ages (25, 28) and persistently infects $CD4^+$ T lymphocytes and salivary glands (3, 12, 14, 22, 24). Although it has been reported that some cases of exanthem subitum are caused by HHV-7 infection (15, 23), the potential association of HHV-7 reactivation in adults with diseases remains to be elucidated.

To clarify the pathological role of virus infection, the identifications of the virus tropism and its receptor are essential. Recently, evidence demonstrating that CD4 is an important component of the binding receptor for HHV-7 as well as human immunodeficiency virus (HIV) has been accumulating. First, down-regulation of surface CD4 is induced by HHV-7 infection (13, 18). Second, HHV-7 infection is inhibited by anti-CD4 monoclonal antibodies (MAbs) and the soluble form of CD4 (18). Third, down-regulation of surface CD4 by ganglioside and phorbol ester inhibits HHV-7 infection (27). Fourth, exposure of $CD4^+$ T lymphocytes or monocytes to HHV-7 interferes with infection by HIV type 1 (HIV-1) and infection by HIV-1 or treatment with recombinant HIV-1 gp120 renders $CD4^+$ T lymphocytes resistant to HHV-7 infection (6, 18). Fifth, radiolabeled HHV-7 specifically binds to HeLa cells expressing surface CD4 (18). Although these data suggest strongly that CD4 is a major component of the receptor for HHV-7, to confirm this possibility it is necessary to examine the infectivity of HHV-7 in originally nonpermissive CD4-negative cells into which the CD4 gene has been transferred. In this study, we used an adenovirus vector to transfer the cDNA for CD4 into CD4-negative or weakly positive cells, because of its high level of gene transfer and low cytotoxicity. The data obtained from the experiments with 13 kinds of cell

lines demonstrate that the CD4 molecule is certainly essential for HHV-7 infection and that HHV-7 can infect and replicate in $CD4^+$ monocytoid and hematopoietic precursor cells as well as T lymphocytes.

The RK strain of HHV-7 was grown in cord blood mononuclear cells that had been stimulated with phytohemagglutinin, as described previously (11). The cell lines were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS). All cell lines proved to be negative for mycoplasma infection.

The generation of replication-defective adenovirus expressing the human *CD4* gene, designated Adex1CACD4, has been described previously (20, 21). Briefly, the expression unit containing the CAG promoter (composed of the cytomegalovirus enhancer and the chicken β-actin promoter), the coding sequence of human CD4, and the rabbit β-globin polyadenylation signal was inserted into an adenovirus cosmid clone with E1 and E3 deleted, pAdex1w. This construct and the EcoT221-digested adenovirus DNA-terminal protein complex were cointroduced into human embryonic kidney 293 cells, and the recombinant adenovirus Adex1CACD4 was isolated. The recombinant adenovirus expressing the *lacZ* gene, designated Adex1CALacZ, was isolated as described above. Adex1CACD4 and Adex1CALacZ were propagated in 293 cells. The titers of the recombinant adenoviruses were determined by plaque-formation assays with 293 cells.

Thirteen cell lines, including T-lymphoid, B-lymphoid, monocytoid, and myeloid cell lines, which were negative for CD4 or only weakly expressed surface CD4, were used. None of these appeared to be susceptible to HHV-7 infection (data not shown). The cells were transduced with Adex1CACD4 or Adex1CALacZ at a multiplicity of infection of approximately 10 and cultured in RPMI 1640 medium supplemented with 10% FCS for 3 days. The expression of surface CD4 following transduction with recombinant adenoviruses was analyzed by flow cytometry. Mock-infected, Adex1CACD4-infected, and Adex1CALacZ-infected cells were stained with the fluorescein isothiocyanate (FITC)-conjugated anti-CD4 MAb Leu3a (Bec-

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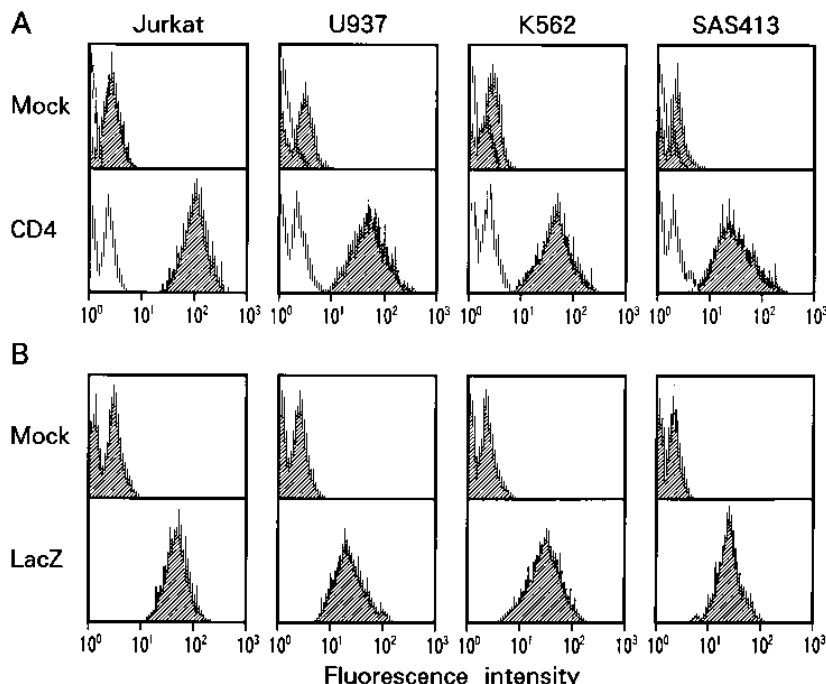
FIG. 1. Expression of surface CD4 and β-galactosidase in cell lines that have been transduced with Adex1CACD4 (A) or Adex1CALacZ (B) at a multiplicity of infection of approximately 10. After 3 days, the levels of expression of surface CD4 (A) and β-galactosidase (B) were examined by flow cytometry, as described in the text. The expression of surface CD4 and β-galactosidase in mock-infected cells is shown in the upper row of each panel. Stainings with the control antibody, FITC-conjugated mouse immunoglobulin G, are shown as open histograms (A).

ton Dickinson, Mountain View, Calif.) and analyzed with a flow cytometer. Cells to be used as an unstained negative control were incubated with FITC-conjugated mouse mono-clonal immunoglobulin G (Becton Dickinson). The expression of β-galactosidase in Adex1CALacZ-transduced cells was ex-amined with fluorescein di-β-D-galactopyranoside (Molecular Probes, Eugene, Oreg.) and a flow cytometer according to the manufacturer's protocol.

The cells (5×10^5) were inoculated with 10^5 50% tissue culture infective doses of HHV-7, suspended in 2 ml of RPMI 1640 medium supplemented with 10% FCS, and cultured in a single well of a 24-well plate. After 5 days, the appearance of cytopathic effect (CPE) was examined with an inverted micro-scope. The replication of HHV-7 was also examined by indirect immunofluorescence assay with 20-fold-diluted HHV-7-sero-positive human serum, as described previously (26). To exam-ine the inhibitory effect of the anti-CD4 MAb on HHV-7 infection, purified Leu3a (Becton Dickinson) was added to the cells at a concentration of 1 μg/ml before inoculation of HHV-7. Semiquantitative PCR assays for the HHV-7 genome were performed as follows. Virus samples, which had been prepared as described above, were frozen, thawed, and soni-cated. Twenty microliters of a 10-fold serially diluted sample was added to a pellet of 2×10^5 HHV-7-uninfected Jurkat cells. DNA was extracted from each sample and amplified for 30 cycles with *Taq* DNA polymerase (Takara Shuzo, Shiga, Japan) and the primers 5′ TATCCCAGCTGTTTTCATAT AGTAAC 3′ and 5′ GCCTTGCGGTAGCACTAGATTTT TTG 3′ (2). The amplified products were electrophoresed on a 2% agarose gel and stained with ethidium bromide. The ex-pected product of HHV-7 DNA that was obtained with these primers was 186 bp.

The representative data of surface CD4 and β-galactosidase expression in mock-infected, Adex1CACD4-transduced, and Adex1CALacZ-transduced cells are shown in Fig. 1. Including the Jurkat, U937, K562, and SAS413 cell lines shown in Fig. 1, all cell lines examined appeared to express surface CD4 and β-galactosidase following transduction with Adex1CACD4 and Adex1CALacZ, respectively (data not shown). Kinetics studies showed that surface CD4 was expressed abundantly after 1 day of recombinant adenovirus infection and that it had been con-tinuously expressed up to at least 12 days after infection.

HHV-7 was inoculated into the cell lines after 3 days of recombinant adenovirus transduction, when surface CD4 was maximally expressed. The appearance of the CPE of HHV-7 was monitored with an inverted microscope. After 5 days of HHV-7 inoculation, Adex1CACD4-transduced Jurkat, U937, K562, and SAS413 cells became larger and formed syncytia, exhibiting typical CPEs of HHV-7 infection (Fig. 2A). More than 40% of these cells appeared to be positive for indirect immunofluorescence with HHV-7-seropositive human se-rum (Fig. 2B). On the other hand, CPEs and positive reac-tions of indirect immunofluorescence were not detected in all Adex1CALacZ-transduced cells which had been inoculated with HHV-7.

To confirm that HHV-7 infected Adex1CACD4-transduced cells through binding to the surface CD4 that was expressed by gene transfer, we examined the inhibitory effect of an anti-CD4 MAb on HHV-7 infection. As shown in Fig. 3, the replication of HHV-7 in Adex1CACD4-transduced SAS413 cells was al-most completely inhibited by addition of the anti-CD4 MAb, suggesting strongly that CD4 is an important component of the receptor for HHV-7. Inhibition of HHV-7 infection by the anti-CD4 MAb was also observed in Adex1CACD4-trans-duced Jurkat, U937, and K562 cells (data not shown). Re-combinant HIV-1 gp120, which binds to CD4 and inhibits HHV-7 infection (18), also inhibited the HHV-7 infection of Adex1CACD4-transduced cells (data not shown).
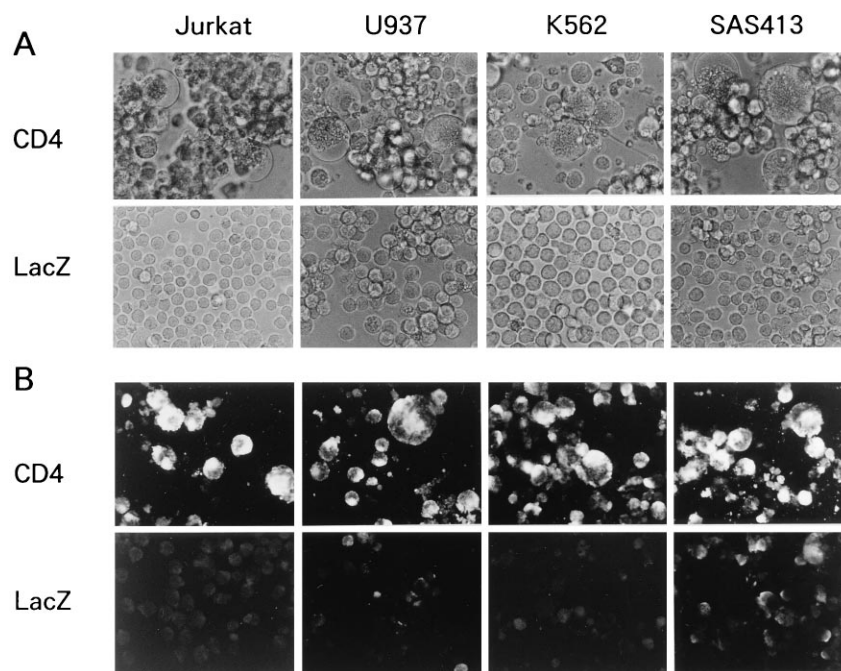
FIG. 2. Replication of HHV-7 in cell lines that have been transduced with Adex1CACD4 or Adex1CALacZ. After 3 days of transduction, the cell lines were inoculated with HHV-7 and cultured for an additional 5 days. The cultured cells were observed with an inverted microscope to detect CPE (A) and analyzed by indirect immunofluorescence with HHV-7-seropositive human serum (B).

The amounts of HHV-7 replicated in Adex1CACD4- and Adex1CALacZ-transduced cells were estimated by semiquantitative PCR. As shown in Fig. 4, *CD4* gene transfer resulted in an approximately 100-fold augmentation of HHV-7 replication in the SAS413 cell line. Similar results were detected in experiments with the Jurkat, U937, and K562 cell lines (data not shown).

In addition to the Jurkat, U937, K562, and SAS413 cell lines, the T-lymphoid, JJHAN, and EU-T02 cell lines showed weak susceptibilities to HHV-7 infection following transfer of the *CD4* gene. In contrast, none of the B-lymphoid cell lines examined showed any susceptibility to HHV-7 infection. A summary of the relative susceptibilities to HHV-7 infection of the 13 cell lines examined following transduction with Adex1CACD4 is given in Table 1.

In this study, we have demonstrated clearly that CD4 is an essential component of the receptor for HHV-7 using a recombinant adenovirus expressing the *CD4* gene. It seems unlikely that the HHV-7 infection of *CD4*-gene-transduced cells resulted from some cellular alterations other than CD4 expression mediated by the recombinant adenovirus, since transduction of the control recombinant adenovirus, which did not contain the *CD4* gene but expressed the *lacZ* gene, showed no effect on HHV-7 infection. In addition, HHV-7 infection was
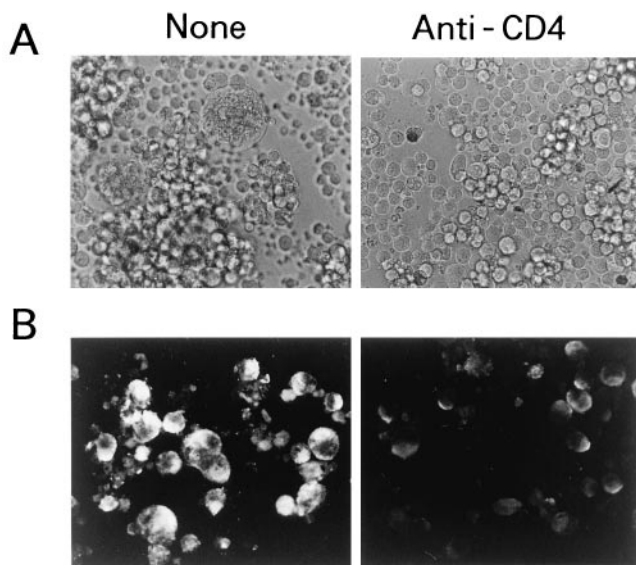

FIG. 3. Effect of an anti-CD4 MAb on HHV-7 replication in *CD4* gene-transduced SAS413 cells. Adex1CACD4-transduced SAS413 cells were treated or not treated (None) with an anti-CD4 MAb, Leu3a, at a concentration of 1 μg/ml before inoculation with HHV-7. After 5 days, the cultured cells were observed with an inverted microscope to detect CPEs (A) and analyzed by indirect immunofluorescence with HHV-7-seropositive human serum (B).
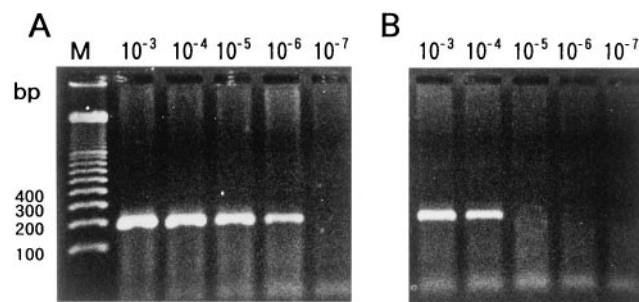

FIG. 4. Semiquantitative PCR analysis of the HHV-7 genome in SAS413 cells transduced with Adex1CACD4 or Adex1CALacZ. Adex1CACD4-transduced (A) and Adex1CALacZ-transduced (B) SAS413 cells were inoculated with HHV-7. After 5 days, the cells were frozen, thawed, and sonicated. Samples were serially diluted 10-fold, and the HHV-7 genome was amplified with primers from 20-μl samples.

TABLE 1. Replication of HHV-7 in various cell lines that had been transduced with Adex1CACD4

Cell line	Cell lineage	Replication of HHV-7[a]
Jurkat	T lymphocyte	+++
EU-T02	T lymphocyte	+
JJHAN	T lymphocyte	+
EU-T01	T lymphocyte	−
Molt-15	T lymphocyte	−
HO8-HT	T lymphocyte	−
Daudi	B lymphocyte	−
Raji	B lymphocyte	−
MY-LCL	B lymphocyte	−
MJ-LCL	B lymphocyte	−
U937	Monocyte	+++
SAS413	Myelocyte	+++
K562	Myeloerythrocyte	+++

[a] Percentages of HHV-7 antigen-positive cells determined by indirect immunofluorescence were as follows: −, less than 10%; +, 10 to 20%; and +++, more than 40%.

almost completely inhibited by the anti-CD4 MAb and transfer of the *CD4* gene did not affect the infectivity of HHV-6, a virus that is closely related to HHV-7 and that infects through binding to a molecule(s) other than CD4 (reference 17 and data not shown).

Although HHV-7 can replicate well in peripheral blood and cord blood CD4$^+$ T lymphocytes, only the Sup-T1, CD4$^+$ T-lymphoid cell line has been shown to be susceptible to HHV-7 infection. Our study has demonstrated that HHV-7 can infect not only T lymphocytes but also various cell lineages, including monocytoid and myeloid cells. It is noteworthy that HHV-7 can infect the CD4$^+$ cell lines established from chronic myelogenous leukemia, a disease of multipotent hematopoietic stem cells. It is well known that the K562 cell line has the potential to differentiate into various hematopoietic cell lineages following suitable stimulation (19). As hematopoietic progenitors appear to express CD4 (16, 29), the present data suggest that HHV-7 can infect hematopoietic precursors and affect hematopoiesis. As with HHV-6 infection in bone marrow, which has been reported recently (4), reactivation of HHV-7 might be one of the causative agents for hematopoietic failure.

It should also be noted that whereas most cell lines expressed surface CD4 by adenovirus vector-mediated gene transfer, some of them were still not susceptible to HHV-7 infection. There are at least two possible explanations for this result. First, binding and penetration of HHV-7 into cells may need some other molecule(s) besides CD4, as reported recently for HIV-1 infection (1, 5, 7–10). Second, HHV-7 replication may depend upon intracellular factors produced by some restricted cells. It is important to clarify the molecules that determine viral tropism, and further studies are required to clarify this point.

The region of CD4 that is required for HHV-7 binding has not yet been mapped. Previous studies have shown that HHV-7 infection is inhibited by an anti-CD4 V1 domain MAb as well as an anti-CD4 V3 domain MAb, whereas an anti-V3 domain MAb could not inhibit HIV-1 infection (18). These data suggest that the binding sites on a CD4 molecule for HHV-7 and HIV-1 are not identical. Further studies using mutated recombinant CD4 molecules may elucidate the precise structures of the HHV-7-binding regions on CD4. In addition, it is essential to identify the HHV-7 structure that binds to the CD4 molecule, since this would shed light on the development of a unique peptide derived from HHV-7, which se-

lectively inhibits HIV-1 binding but does not affect the functions of CD4$^+$ cells.

REFERENCES

1. **Alkhatib, G., C. Combadiere, C. C. Broder, Y. Feng, P. E. Kennedy, P. M. Murphy, and E. A. Berger.** 1996. CC CKR5: a RANTES, MIP-1α, MIP-1β receptor as a fusion cofactor for macrophage-tropic HIV-1. Science **272:** 1955–1958.
2. **Berneman, Z. N., D. V. Ablashi, G. Li, M. Eger-Fletcher, M. S. Reitz, Jr., C.-L. Hung, I. Brus, A. L. Komaroff, and R. C. Gallo.** 1992. Human herpesvirus 7 is a T-lymphotropic virus and is related to, but significantly different from, human herpesvirus 6 and human cytomegalovirus. Proc. Natl. Acad. Sci. USA **89:**10552–10556.
3. **Black, J. B., N. Inoue, K. Kite-Powell, S. Zaki, and P. E. Pellett.** 1993. Frequent isolation of human herpesvirus 7 from saliva. Virus Res. **29:**91–98.
4. **Carrigan, D. R., and K. K. Knox.** 1994. Human herpesvirus 6 (HHV-6) isolation from bone marrow: HHV-6-associated bone marrow suppression in bone marrow transplant patients. Blood **84:**3307–3310.
5. **Choe, H., M. Farzan, Y. Sun, N. Sullivan, B. Rollins, P. D. Ponath, L. Wu, C. R. Mackay, G. LaRosa, W. Newman, N. Gerard, C. Gerard, and J. Sodroski.** 1996. The β-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. Cell **85:**1135–1148.
6. **Crowley, R. W., P. Secchiero, D. Zella, A. Cara, R. C. Gallo, and P. Lusso.** 1996. Interference between human herpesvirus 7 and HIV-1 in mononuclear phagocytes. J. Immunol. **156:**2004–2008.
7. **Deng, H., R. Liu, W. Ellmeier, S. Choe, D. Unutmaz, M. Burkhart, P. Di Marzio, S. Marmon, R. E. Sutton, C. M. Hill, C. B. Davis, S. C. Peiper, T. J. Schall, D. R. Littman, and N. R. Landau.** 1996. Identification of a major co-receptor for primary isolates of HIV-1. Nature **381:**661–666.
8. **Doranz, B. J., J. Rucker, Y. Yi, R. J. Smyth, M. Samson, S. C. Peiper, M. Parmentier, R. G. Collman, and R. W. Doms.** 1996. A dual-tropic primary HIV-1 isolate that uses fusin and the β-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. Cell **85:**1149–1158.
9. **Dragic, T., V. Litwin, G. P. Allaway, S. R. Martin, Y. Huang, K. A. Nagashima, C. Cayanan, P. J. Maddon, R. A. Koup, J. P. Moore, and W. A. Paxton.** 1996. HIV-1 entry into CD4$^+$ cells is mediated by the chemokine receptor CC-CKR-5. Nature **381:**667–673.
10. **Feng, Y., C. C. Broder, P. E. Kennedy, and E. A. Berger.** 1996. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science **272:**872–877.
11. **Frenkel, N., E. C. Schirmer, L. S. Wyatt, G. Katsafanas, E. Roffman, R. M. Danovich, and C. H. June.** 1990. Isolation of a new herpesvirus from human CD4$^+$ T cells. Proc. Natl. Acad. Sci. USA **87:**748–752.
12. **Frenkel, N., and L. S. Wyatt.** 1992. HHV-6 and HHV-7 as exogenous agents in human lymphocytes. Dev. Biol. Stand. **76:**259–265.
13. **Furukawa, M., M. Yasukawa, Y. Yakushijin, and S. Fujita.** 1994. Distinct effects of human herpesvirus 6 and human herpesvirus 7 on surface molecule expression and function of CD4$^+$ T cells. J. Immunol. **152:**5768–5775.
14. **Hidaka, Y., Y. Liu, M. Yamamoto, R. Mori, C. Miyazaki, K. Kusuhara, K. Okada, and K. Ueda.** 1993. Frequent isolation of human herpesvirus 7 from saliva samples. J. Med. Virol. **40:**343–346.
15. **Hidaka, Y., K. Okada, K. Kusuhara, C. Miyazaki, K. Tokugawa, and K. Ueda.** 1994. Exanthem subitum and human herpesvirus 7 infection. Pediatr. Infect. Dis. J. **13:**1010–1011.
16. **Louache, F., N. Debili, A. Marandin, L. Coulombel, and W. Vainchenker.** 1994. Expression of CD4 by human hematopoietic progenitors. Blood **84:** 3344–3355.
17. **Lusso, P., R. C. Gallo, S. E. DeRocco, and P. D. Markham.** 1989. CD4 is not the membrane receptor for HHV-6. Lancet **i:**730.
18. **Lusso, P., P. Secchiero, R. W. Crowley, A. Garzino-Demo, Z. N. Berneman, and R. C. Gallo.** 1994. CD4 is a critical component of the receptor for human herpesvirus 7: interference with human immunodeficiency virus. Proc. Natl. Acad. Sci. USA **91:**3872–3876.
19. **Marie, J. P., C. A. Ijaguirre, C. I. Civin, J. Mirro, and E. A. McCulloch.** 1981. The presence within single K562 cells of erythropoietic and granulopoietic differentiation markers. Blood **58:**708–711.
20. **Miyake, K., T. Tohyama, and T. Shimada.** Two step gene transfer using an adenovirus vector carrying the CD4 gene and HIV vectors. Hum. Gene Ther., in press.
21. **Miyake, S., M. Makimura, Y. Kanegae, S. Harada, Y. Sato, K. Takamori, C. Tokuda, and I. Saito.** 1996. Efficient generation of recombinant adenoviruses using adenovirus DNA-terminal protein complex and a cosmid bearing the full-length virus genome. Proc. Natl. Acad. Sci. USA **93:**1320–1324.
22. **Sada, E., M. Yasukawa, C. Ito, A. Takeda, T. Shiosaka, H. Tanioka, and S.**

Fujita. 1996. Detection of human herpesvirus 6 and human herpesvirus 7 in the submandibular gland, parotid gland, and lip salivary gland by PCR. J. Clin. Microbiol. **34:**2320–2321.

23. **Tanaka, K., T. Kondo, S. Torigoe, S. Okada, T. Mukai, and K. Yamanishi.** 1994. Human herpesvirus 7: another causal agent for roseola (exanthem subitum). J. Pediatr. **125:**1–5.

24. **Wyatt, L. S., and N. Frenkel.** 1992. Human herpesvirus 7 is a constitutive inhabitant of adult human saliva. J. Virol. **66:**3206–3209.

25. **Wyatt, L. S., W. J. Rodriguez, N. Balachandran, and N. Frenkel.** 1991. Human herpesvirus 7: antigenic properties and prevalence in children and adults. J. Virol. **65:**6260–6265.

26. **Yakushijin, Y., M. Yasukawa, and Y. Kobayashi.** 1991. T-cell immune response to human herpesvirus-6 in healthy adults. Microbiol. Immunol. **35:** 655–660.

27. **Yasukawa, M., Y. Inoue, E. Sada, Y. Yakushijin, M. Furukawa, and S. Fujita.** 1995. CD4 down-modulation by ganglioside and phorbol ester inhibits human herpesvirus 7 infection. J. Gen. Virol. **76:**2381–2385.

28. **Yoshikawa, T., Y. Asano, I. Kobayashi, T. Nakashima, T. Yazaki, S. Suga, T. Ozaki, L. S. Wyatt, and N. Frenkel.** 1993. Seroepidemiology of human herpesvirus 7 in healthy children and adults in Japan. J. Med. Virol. **41:**319–323.

29. **Zauli, G., G. Furlini, M. Vitale, M. C. Re, D. Gibellini, L. Zamai, G. Visani, P. Borgatti, S. Capitani, and M. La Place.** 1994. A subset of human CD34$^+$ hematopoietic progenitors express low levels of CD4, the high-affinity receptor for human immunodeficiency virus-type 1. Blood **84:**1896–1905.