JOURNAL OF VIROLOGY, July 1989, p. 3161–3163
0022-538X/89/073161-03$02.00/0
Copyright © 1989, American Society for Microbiology
Vol. 63, No. 7

Predominant CD4 T-Lymphocyte Tropism of Human Herpesvirus 6-Related Virus

KAZUO TAKAHASHI,[1,2] SHUNRO SONODA,[2] KAZUHIRO HIGASHI,[1] TOSHIO KONDO,[3] HIROHIKO TAKAHASHI,[4] MICHIAKI TAKAHASHI,[1] AND KOICHI YAMANISHI[1]*

Department of Virology, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565,[1] Department of Virology, Faculty of Medicine, Kagoshima University, Kagoshima 890,[2] Kondo Clinic, Toyonaka, Osaka,[3] and Onoda Municipal Hospital, Onoda, Yamaguchi,[4] Japan

Received 28 November 1988/Accepted 13 March 1989

Human herpesvirus 6 (HHV-6)-related virus was isolated from CD4$^+$ CD8$^-$ and CD3$^+$ CD4$^+$ mature T lymphocytes but could not be isolated from CD4$^-$ CD8$^+$, CD4$^-$ CD8$^-$, and CD3$^-$ T cells in the peripheral blood of exanthem subitum patients. HHV-6-related virus predominantly infected CD4$^+$ CD8$^+$, CD4$^+$ CD8$^-$, and CD3$^+$ CD4$^+$ cells with mature phenotypes and rarely infected CD4$^-$ CD8$^+$ cells from cord blood mononuclear cells, which suggested predominant CD4 mature T-lymphocyte tropism of HHV-6-related virus.

A novel human herpesvirus was isolated from patients with lymphoproliferative disorders (10) and named human B-lymphotropic virus (HBLV); since that time, viruses closely related to or genetically indistinguishable from human B-lymphotropic virus have been isolated mainly from patients with acquired immunodeficiency syndrome (2, 3, 5, 12). Since these viruses were subsequently shown to have diverse cellular tropism in vitro, it was suggested that human B-lymphotropic virus be designated human herpesvirus 6 (HHV-6) (7). However, there remains controversy as to the cellular tropism both in cord blood mononuclear cells (CBMC) and in some human hematopoietic cell lines (1, 3, 6, 10).

We recently isolated from peripheral blood mononuclear cells of patients a causative agent of exanthem subitum (ES) that was closely related, antigenically and morphologically, to HHV-6 (11, 13). Until this virus can be completely characterized by molecular biological techniques, we tentatively refer to it as HHV-6-related virus. In this study, we investigated the in vivo cellular tropism of this virus in ES patients and further examined its in vitro cellular tropism for CBMC.

Peripheral blood mononuclear cells of ES patients in the febrile phase were separated by Ficoll-Paque (Pharmacia, Inc., Piscataway, N.J.) and incubated in gelatin-coated dishes for 2 h in Dulbecco modified minimum essential medium supplemented with 10% fetal calf serum as described elsewhere (4). Nonadherent cells were pipetted off gently several times with Dulbecco modified minimum essential medium prewarmed at 37°C, and adherent monocytes were removed by 5 mM EDTA in phosphate-buffered saline. These monocytes were simply cocultured, and nonadherent cells were cocultured in serial 10-fold dilutions with 10^6 phytohemagglutinin (PHA)-P (Honen Oil Co., Tokyo, Japan)-stimulated CBMC in complete medium (RPMI 1640 supplemented with 10% fetal calf serum, 5 μg of PHA-P per ml, and 0.1 U of recombinant human interleukin-2 [IL-2; Takeda Chemical Industries, Tokyo, Japan]). At 14 days after cultivation, the virus-infected cells were detected with convalescent serum of an ES patient by an immunofluorescence test as described previously (13). The convalescent serum has been confirmed to react with HHV-6 isolated by Lopez et al. (5).

HHV-6-related virus was isolated from nonadherent cells but not from adherent monocytes (Table 1). The frequency of virus-infected cells was at least 10^3 in patient 1. To further characterize the in vivo cellular tropism of the virus, the CD4$^+$ CD8$^-$, CD4$^-$ CD8$^+$, CD4$^-$ CD8$^-$, CD3$^+$ CD4$^+$, and CD3$^-$ cell subpopulations were segregated from peripheral blood mononuclear cells of six ES patients by a cell sorter (FACStar; Becton Dickinson and Co., Paramus, N.J.), using fluorescein isothiocyanate-conjugated CD4 and phycoerythryn-conjugated CD8 and CD3 monoclonal antibodies. Contamination of CD8$^+$ cells in the CD4$^+$ CD8$^-$ population and of CD4$^+$ cells in the CD4$^-$ CD8$^+$ population was less than 0.01%, and that of CD4$^+$ or CD8$^+$ cells in the CD4$^-$ CD8$^-$ cell fraction was less than 1%, as analyzed by FACScan (Becton Dickinson).

Viable lymphocytes segregated by serial 10-fold dilution were cocultured with CBMC, and viral antigens in virus-infected cells were detected as described above. HHV-6-related virus was consistently isolated from CD4$^+$ CD8$^-$ cells in four patients and from CD3$^+$ CD4$^+$ cells in two patients, but none was isolated from CD4$^-$ CD8$^+$, CD4$^-$ CD8$^-$, or CD3$^-$ cells (Table 2). Since the mononuclear cells of patients 4, 5, and 7 included B cells at 18, 10, and 16%, respectively, as determined by examination with NU-B2 monoclonal antibody (CD20; Nichirei Corp., Tokyo, Japan), the CD4$^-$ CD8$^-$ cells are assumed to have included some proportion of B cells. The frequency of virus-infected CD4$^+$ CD8$^-$ cells was at least $1/10^4$ in three patients and $1/10^5$ in one patient. These results indicate that HHV-6-related virus predominantly infects CD4$^+$ CD8$^-$ and CD3$^+$ CD4$^+$ T lymphocytes with mature phenotypes (9) in vivo, although only a limited number of cells (10^5) could be examined.

We therefore attempted to elucidate the characteristics of target cell populations for this virus by infecting the subpopulations of CBMC. CD4$^+$ CD8$^+$, CD4$^+$ CD8$^-$, CD4$^-$ CD8$^+$, CD3$^+$ CD4$^+$, CD3$^-$ CD4$^+$, and CD3$^-$ CD4$^-$ cells were isolated from three samples of PHA-stimulated CBMC by a cell sorter. Cells of each subpopulation (10^6) were inoculated with 0.1 ml of cell-free virus (HST strain; 10^3 50% tissue culture infective doses) isolated from an ES patient and incubated at 37°C for 1 h; then 0.9 ml of complete medium was added. Seven days after culture, dead cells were par-

* Corresponding author.

TABLE 1. Isolation of HHV-6-related virus from nonadherent mononuclear cells but not from adherent monocytes of ES patients

Patient	Total no. of mononuclear cells (10^6)	Isolation of virus from:						
		Nonadherent mononuclear cells						Adherent monocytes (no. of cells, 10^5)
		10^6	10^5	10^4	10^3	10^2	10^1	
1	2.8	+	+	+	+	−	−	− (2.5)
2	2.3	+	−	−	−	−	−	− (1.9)
3	3.0	+	−	−	−	−	−	− (3.1)

tially removed by Ficoll-Paque gradient centrifugation, and virus-infected cells were applied with diluted patient convalescent serum and each monoclonal antibody to surface markers of lymphocytes (OKT3 [CD3], OKT4 [CD4], and OKT8 [CD8]; Ortho Pharmaceuticals, Raritan, N.J.) and anti-Tac [anti-IL-2 receptor; CD25), followed by reaction with fluorescein isothiocyanate-conjugated goat anti-human immunoglobulin G and phycoerythrin-conjugated rabbit anti-mouse immunoglobulin G antibodies (Organon Teknika, Malvern, Pa.). The cells were examined for surface markers by two-color flow cytometric analysis. Debris and dead cells were excluded from the analysis by a scatter setting system. Approximately 80% of the cultured cells were positive for virus antigen in $CD4^+$ $CD8^+$, $CD4^+$ $CD8^-$, and $CD3^+$ $CD4^+$ target cells, and almost all of virus-positive cells in these subpopulations expressed CD4 and Tac antigen (Table 3). These infected cells exhibited notable cytopathic effect (balloonlike). On the other hand, only 1.3% of the cultured cells in $CD4^-$ $CD8^+$ target cells were positive for the virus, although 33.3% were $CD4^+$ (Table 3). Furthermore, almost all virus-positive cells in the $CD4^-$ $CD8^+$ subset expressed the CD4 antigen (Table 3). This result is in contrast to the low CD8 expression (7.2%) in the virus-positive cells among $CD4^+$ $CD8^-$ target cells. In $CD4^+$ $CD8^+$ and $CD4^-$ $CD8^+$ cells, most of the virus-infected cells were CD3 positive; the proportion was lower in $CD4^+$ $CD8^-$ and $CD3^+$ $CD4^+$ cells. Since $CD3^-$ cells did not proliferate in complete medium and gradually died, the number of viable cells was too small to enable analysis of surface markers. In any event, virus antigen-positive viable $CD3^-$ cells have not been observed

TABLE 2. Isolation of HHV-6-related virus from lymphocyte subpopulations of ES patients

Patient	Subpopulation	Isolation of virus from given no. of cells cocultured with CBMC			
		10^5	10^4	10^3	10^2
4	$CD4^+$ $CD8^-$	+	+	−	−
	$CD8^+$ $CD4^-$	−	−	−	−
	$CD4^-$ $CD8^-$	−	−	−	−
5	$CD4^+$ $CD8^-$	+	−	−	−
	$CD8^+$ $CD4^-$	−	−	−	−
	$CD4^-$ $CD8^-$	−	−	−	−
6	$CD4^+$ $CD8^-$	+	+	−	−
	$CD8^+$ $CD4^-$	−	−	−	−
7	$CD4^+$ $CD8^-$	+	+	−	−
	$CD8^+$ $CD4^-$	−	−	−	−
	$CD4^-$ $CD8^-$	−	−	−	−
8	$CD4^+$ $CD3^+$	+	−	−	−
	$CD3^-$	−	−	−	−
9	$CD4^+$ $CD3^+$	+	+	−	−
	$CD3^-$	−	−	−	−

TABLE 3. Infection of lymphocyte subpopulations with HHV-6-related virus

Antigen	Level of infection[a] in given subpopulation			
	$CD4^+$ $CD8^+$	$CD4^+$ $CD8^-$	$CD4^-$ $CD8^+$	$CD3^+$ $CD4^+$
HHV-6-RV	83.6[b]	85.1	1.3	78.0
CD3	82.9, 85.2	46.8, 45.6	87.1, 94.8	63.1, 66.6
CD4	88.2, 99.1	99.7, 99.6	33.3, 97.7	95.3, 99.9
CD8	84.1, 96.2	20.1, 7.2	96.9, 98.7	47.1, 57.8
Tac	84.0, 94.4	90.3, 97.4	8.9, 36.8	76.6, 91.5

[a] First number is percent given antigen-positive cells; second number is percentage of surface marker-positive cells among HHV-6-related virus-infected (HHV-6-RV) cells. $CD3^-$ cells were not culturable.

[b] Mean value from three samples.

so far. Since the virus antigens can be detected only on the cell surface membrane in this fluorescence-activated cell sorter analysis, we examined the possibility that there were virus-infected cells in the $CD4^-$ $CD8^+$ subpopulation that expressed the viral antigens only in the cytoplasm and nucleus. The frequency of virus-infected cells in fixed cell preparations as determined by immunofluorescence assay was similar to that obtained by cell sorter analysis (Fig. 1).

The results of this study indicate that HHV-6-related virus has selective tropism for CD4 lymphocytes, which partially supports the results of recent reports (2, 6). Although the

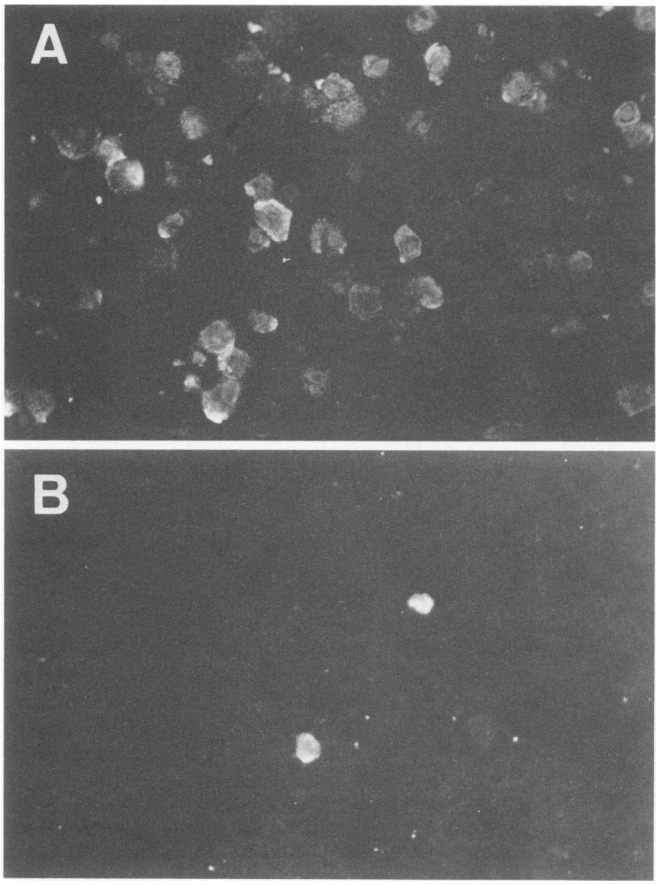

FIG. 1. Immunofluorescence of HHV-6-related virus-infected cells in target $CD4^+$ $CD8^-$ (A) and $CD4^-$ $CD8^+$ (B) cells stained with convalescent serum of an ES patient and fluorescein-conjugated goat anti-human immunoglobulin G.

HST strain used in these experiments is closely related, if not identical, to HHV-6, its cell tropism differed from that reported for HHV-6 (10). In ES patients, the virus could not be isolated from B cells. Furthermore, the finding that the virus can infect cord blood B cells was not obtained by assaying with an complement-anticomplement method to exclude nonspecific binding of secondary antibody, anti-human immunoglobulin G, to surface immunoglobulin on B cells (data not shown).

Lusso et al. (6, 7) reported that HHV-6-infected T cells lack the surface membrane TCR/CD3 complex and IL-2 receptor and partially coexpress the CD4 and CD8 antigens. HHV-6 was shown to infect predominantly CD3-depleted immature T cells but not $CD3^+$ mature cells. Our results differ considerably. HHV-6-related virus infected both $CD4^+$ $CD8^+$ blastic cells and $CD4^+$ $CD8^-$ mature cells. The former markers are characteristic of the immature cortical thymic T cells. However, the virus-infected cells in the $CD4^+$ $CD8^+$ subpopulation predominantly expressed CD3 antigen and IL-2 receptor, which are the markers of mature T cells, and those in $CD4^+$ $CD8^-$ and $CD4^-$ $CD8^+$ cells also expressed CD3 antigen, although to a lesser extent in the former cells. Furthermore, $CD3^+$ $CD4^+$ cells of PHA-stimulated CBMC were highly susceptible to virus infection, whereas $CD3^-$ $CD4^+$ and $CD3^-$ $CD4^-$ cells did not respond to PHA and IL-2, resulting in cell death. Therefore, most of the susceptible cells reside within the $CD3^+$ $CD4^+$ mature T cells, not in $CD3^-$ immature T cells. This result agrees with those reported by Downing et al. (3). The discrepancy regarding the cell tropism of HHV-6 and the virus studied in this work may be due to conditions of cell culture, namely, the presence or absence of PHA and IL-2 in the culture medium. PHA and IL-2 are known to induce IL-2 receptor and activate T cells to proliferate, expressing IL-2 receptor and CD3 antigen. Alternatively, the discrepancy may be due to the difference in HHV-6 strains studied; this is unlikely to be the case, however, since the HHV-6 strains used are antigenically and morphologically similar, if not identical, to the original human B-lymphotropic virus (5, 10, 13). The reason for the loss of CD3 antigen in a $CD3^+$ $CD4^+$ subpopulation (36.9% negative) is not clear. Since the ratio of CD3-positive cells was relatively variable, depending on culture well, this loss may reflect the apparent loss of CD3 antigen.

PHA-stimulated $CD4^-$ $CD8^+$ cells were not particularly susceptible to virus infection (1.3%) despite a relatively high proportion of $CD4^+$ cells (33.3%). The reason why only 1.3% of the cells were infected in comparison with approximately 80% of cells for all $CD4^+$ cells in a $CD4^+$ $CD8^-$ subpopulation is unknown. CD4 antigen may be gradually induced in its subpopulation by PHA and IL-2 in the culture such that the virus cannot replicate sufficiently in $CD4^+$ cells. Alternatively, factors other than CD4 antigen may be involved. For example, IL-2 receptor was scarcely expressed in a $CD4^-$ $CD8^+$ subpopulation (8.9%). Since noninfected $CD4^-$ $CD8^+$ cells also rarely expressed IL-2 receptor and almost all virus-infected cells expressed virus antigens on the surface membrane, it is unlikely that the virus suppressed expression of the IL-2 receptor. This resistance of $CD4^-$ $CD8^+$ cells to the virus may be due in part to the lack of cell proliferation caused by unresponsiveness to IL-2.

Our finding that HHV-6-related virus is selectively tropic for $CD4^+$ T lymphocytes both in vivo and in vitro may have some implications for the involvement of HHV-6 in the pathogenesis of CD4 T-cell disorders such as human immunodeficiency virus and human T-cell leukemia virus type I infections. HHV-6 infection or reactivation in the immunosuppressive state of these diseases may modify or exacerbate the diseases.

We are grateful to Kohjiro Nakamura for excellent technical assistance, to Takashi Uchiyama for providing anti-Tac monoclonal antibody, and to Chifumi Yamada and Stephanie M. Mizsak for preparing the manuscript.

LITERATURE CITED

1. **Ablashi, D. V., S. Z. Salahuddin, S. F. Josephs, F. Imam, P. Lusso, R. C. Gallo, C. Hung, J. Lemp, and P. D. Markham.** 1987. HBLV (or HHV-6) in human cell lines. Nature (London) **329:**207.
2. **Agut, H., D. Guetard, H. Collandre, C. Dauguet, L. Montagnier, J. Miclea, H. Baurmann, and A. Gessain.** 1988. Concomitant infection by human herpesvirus 6, HTLV-I, and HIV-2. Lancet **i:**712.
3. **Downing, R. G., N. Sewankambo, D. Serwadda, R. Honess, D. Crawford, R. Jarrett, and B. E. Griffin.** 1987. Isolation of human lymphotropic herpesviruses from Uganda. Lancet **ii:**390.
4. **Hassan, N. F., D. E. Campbell, and S. D. Douglas.** 1986. Purification of human monocytes on gelatin-coated surfaces. J. Immunol. Methods **95:**273–276.
5. **Lopez, C., P. Pellett, J. Stewart, C. Goldsmith, K. Sanderlin, J. Black, D. Warfield, and P. Feorino.** 1988. Characteristics of human herpesvirus-6. J. Infect. Dis. **157:**1271–1273.
6. **Lusso, P., P. D. Markham, E. Tschachler, F. M. Veronese, S. Z. Salahuddin, D. V. Ablashi, S. Pahwa, K. Krohn, and R. C. Gallo.** 1988. In vitro cellular tropism of human B-lymphotropic virus (human herpesvirus-6). J. Exp. Med. **167:**1659–1670.
7. **Lusso, P., S. Z. Salahuddin, D. V. Ablashi, R. C. Gallo, F. D. Veronese, and P. D. Markham.** 1987. Diverse tropism of human B-lymphotropic virus (human herpesvirus 6. Lancet **ii:**743–744.
8. **Miyoshi, I., H. Taguchi, I. Kubonishi, S. Yoshimoto, Y. Ohtsuki, Y. Shiraishi, and T. Akagi.** 1982. Type C virus-producing cell lines derived from adult T cell leukemia. Gann Monogr. **28:** 219–228.
9. **Reinherz, E. L., P. C. Kung, G. Goldstein, R. H. Levey, and S. F. Schlossman.** 1980. Discrete stages of human intrathymic differentiation: analysis of normal thymocytes and leukemic lymphoblasts of T-cell lineage. Proc. Natl. Acad. Sci. USA **77:**1588–1592.
10. **Salahuddin, S. Z., D. V. Ablashi, P. D. Markham, S. F. Josephs, S. Sturzenegger, M. Kaplan, G. Halligan, P. Biberfeld, F. Wong-Staal, B. Kramarsky, and R. C. Gallo.** 1986. Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. Science **234:**596–601.
11. **Takahashi, K., S. Sonoda, K. Kawakami, K. Miyata, T. Oki, H. Nagata, T. Okuno, and K. Yamanishi.** 1988. Human herpesvirus 6 and exanthem subitum. Lancet **i:**1463.
12. **Tedder, R. S., M. Briggs, C. H. Cameron, R. Honess, D. Robertson, and H. Whittle.** 1987. A novel lymphotropic herpesvirus. Lancet **ii:**390–392.
13. **Yamanishi, K., T. Okuno, K. Shiraki, M. Takahashi, T. Kondo, Y. Asano, and T. Kurata.** 1988. Identification of human herpesvirus-6 as a causal agent for exanthem subitum. Lancet **i:** 1065–1067.