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Biological and Molecular Characteristics of Human Herpesvirus 7: *In Vitro* Growth Optimization and Development of a Syncytia Inhibition Test

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Two isolates of human herpesvirus 7 (HHV-7) were recovered from phytohemagglutinin-activated peripheral blood mononuclear cells of a patient with chronic fatigue syndrome and of a healthy blood donor. A genetic polymorphism between the two isolates was detected by Southern blot analysis using a novel HHV-7 genomic clone (pVL8) as a probe. We developed optimized conditions for the *in vitro* propagation of HHV-7 by using enriched populations of activated CD4$^+$ T lymphocytes derived from normal peripheral blood, resulting in the production of high-titered extracellular virus (>10^6 cell culture infectious doses/ml). *Bona fide* syncytia formation was documented both in normal CD4$^+$ T lymphocytes and in the Sup-T1 CD4$^+$ T-cell line following infection with high-titered HHV-7. To identify neutralizing antibodies to HHV-7, a syncytia-inhibition test was developed. Variable titers of syncytia-neutralizing antibodies were detected in all the human sera tested, thus confirming the high prevalence of HHV-7 in the human population. © 1994 Academic Press, Inc.

Human herpesvirus 6 (HHV-6) (*1–4*) and HHV-7 (*5, 6*), the last discovered members of the *Herpesviridae* family, constitute a new subfamily of T-lymphotropic human herpesviruses (*2, 3, 5–8*). HHV-6 was discovered in 1986 from the peripheral blood of immunosuppressed patients affected by lymphoproliferative disorders and, in two cases, by the acquired immunodeficiency syndrome (AIDS) (*1*). HHV-7 was identified for the first time in 1990 in CD4$^+$ T cells of a healthy individual (*5*) and, subsequently, in peripheral blood mononuclear cells (PBMC) of a patient with chronic fatigue syndrome (CFS) (*6*), as well as in the saliva of healthy adults (*9*). Molecular, immunologic, and biologic studies demonstrated that, among the human herpesviruses, HHV-6 and HHV-7 are most closely related; yet the two viruses differ significantly. Analysis by DNA hybridization showed that the molecular divergence between HHV-6 and HHV-7 is far greater than that documented between the two major HHV-6 variants, A and B (*5–7*). A limited degree of genetic homology with human cytomegalovirus (hCMV) supports the classification of HHV-7, together with HHV-6, as a member of the β-herpesvirus subfamily (*7*). Seroepidemiological studies have demonstrated that infections by HHV-6 (*10, 11*) and HHV-7 (*7, 12*) are widely diffused in the general population and that seroconversion commonly occurs prior to 4 years of age (*12, 13*). Like most herpesviruses, both HHV-6 and HHV-7 are likely to persist in the host in a latent state after primary infection. Whereas HHV-6 has been etiologically linked to *exanthem subitum* (*14*), a benign acute illness of infancy, and to severe pathology in immunosuppressed individuals (*15–17*), no evidence for an association of HHV-7 with human disease has yet emerged. We have recently demonstrated that HHV-7 uses CD4 as an essential component of its cellular membrane receptor (*18*). This observation has raised the question whether HHV-7 can interfere with human immunodeficiency virus (HIV), the causative agent of AIDS (*19, 20*), which uses the same membrane receptor on susceptible cells (*21, 22*).

We recovered two cytopathic viral isolates from cultured PBMC of a patient with CFS (JB) and of a healthy blood donor (AL), respectively. In contrast, no evidence of cytopathic effect was seen in more than 200 samples of normal adult human PBMC, which were routinely cultured in our laboratory during the past 4 years, as well as in PBMC from 4 additional patients with CFS, which were cultured under the same conditions to attempt viral isolation. Mononuclear cells were purified from heparinized blood by Ficoll–Hypaque (Pharmacia) gradient centrifugation, washed thrice with phosphate-buffered saline, and cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1 μg/ml of purified phytohemagglutinin (PHA) (Wellcome). Twenty-four hours later, the cells were washed and recultured in complete medium in the presence of partially purified, native human interleukin 2 (IL-2) (Boehringer Mannheim) at 10 U/ml. After 6–10 days of culture, the cells started to exhibit a cytopathic effect consisting of enlarged, refractile, short-lived cells, sometimes exhibiting the typical features of giant multinucleated cells. At that time, the cells

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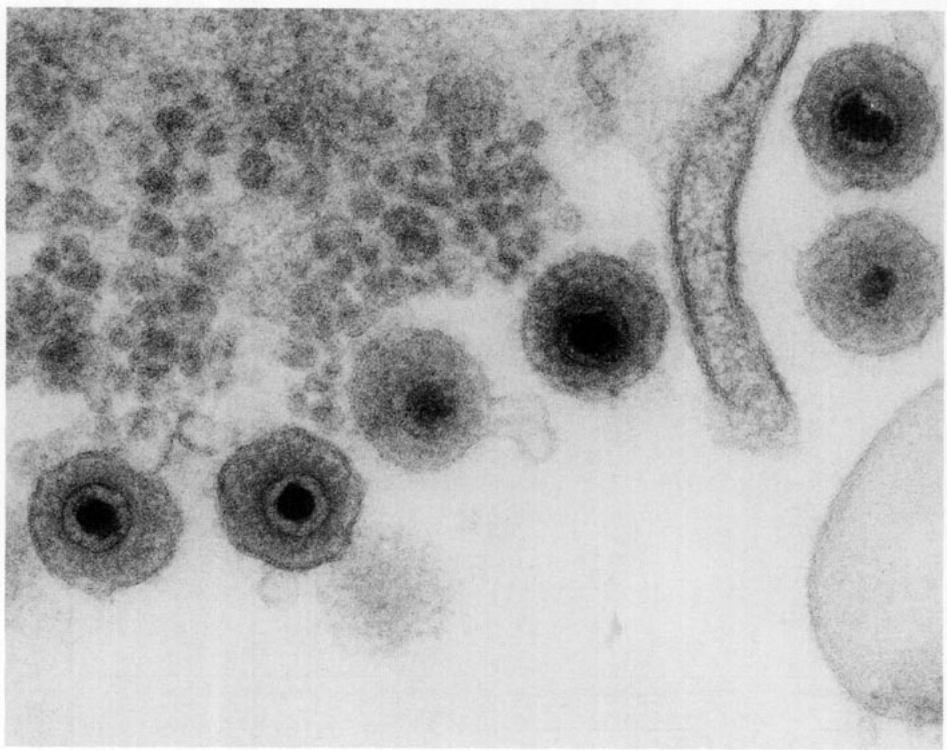

FIG. 1. Electron microscopy of peripheral blood $CD4^+$ T cells infected with HHV-7_{JB}. Mature virus particles in the extracellular space (magnification: ×95,000).

were cocultured with PHA-stimulated umbilical cord blood mononuclear cells (CBMC). Five to 8 days later, large cells again started to appear. Exposure of PHA-stimulated CBMC to cell-free supernatants from infected cultures also resulted in the appearance of large cells similar to those observed in both primary and secondary cultures.

Transmission electron microscopy was performed on glutaraldehyde-fixed thin sections from cells infected with isolates JB and AL. This analysis revealed the presence of viral particles with the characteristic morphological features of herpesviruses (*23*) (Fig. 1). Both intranuclear and cytoplasmic virions at different stages of maturation were visible. Mature, enveloped virions, approximately 200 nm in diameter, were released into the extracellular space. They contained an electron-dense core surrounded by the capsid membrane, a tegument and an external envelope, sometimes exhibiting evident spike-shaped projections on the external surface. To characterize these herpesvirus isolates, acetone-fixed infected cells were analyzed by indirect immunofluorescence (IF) with murine monoclonal antibodies (MAbs), as previously described (*8*). Fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G (IgG) antiserum was used as a second reagent. The two isolates exhibited the typical reactivity pattern of HHV-7 (*7, 12*). Infected cells were recognized by two MAbs, 9A5D12 (p41) and 12B3G4 (p135), originally developed against HHV-6 (*24*), but known to cross-react with HHV-7 (*7, 12*). In contrast, no reactivity was seen with other MAbs specific for HHV-6, (2D6 and 13D6 [gp82/105], 6A5G3 [gp116/65/54]) (*24*), or with MAbs specific for hCMV (9220 [early nuclear protein], 9221 [late nuclear protein]) (Dupont).

To confirm that the JB and AL viruses were HHV-7, polymerase chain reaction (PCR) amplification was carried out, using primers specific for HHV-6 or HHV-7, as reported elsewhere (*7, 25*). Primers derived from a highly conserved region of the HHV-6 genome did not amplify DNA extracted from cells infected with HHV-7_{AL} or HHV-7_{JB} (Fig. 2A). However, the expected 186-bp PCR product was amplified from both isolates after a single round of amplification using a pair of HHV-7-specific primers, HV7 and HV8 (Fig. 2B). The specificity of the reaction was confirmed by Southern blot hybridization of the PCR product with the internal, ^{32}P-labeled oligodeoxynucleotide probe, HV12 (data not shown).

To definitively establish the identity of the JB and AL isolates, we performed Southern blot hybridizations using a panel of novel recombinant DNA probes cloned in pBluescript plasmid (Stratagene) from HHV-7_{JI} (Z. N. Berneman, manuscript in preparation). High-molecular-weight DNA, extracted from Sup-T1 cells infected with isolates AL, JB, and JI (*6*), was digested with different restriction endonucleases and analyzed by Southern blot, as previously described (*7*). Genomic DNA from cells

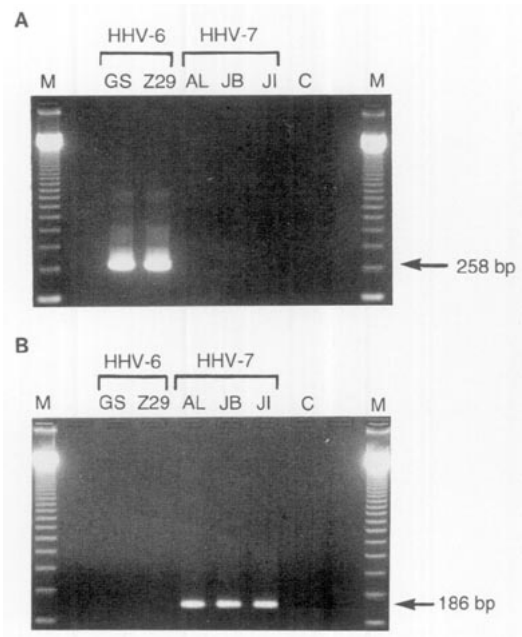

FIG. 2. PCR amplification of DNA from HHV-6- and HHV-7-infected cells. Ethidium bromide-staining to visualize: (A) the products of PCR amplification performed with two nested pairs of primers specific for HHV-6. Outer primer pair: EX1 (5′-GCGTTTTCAGTGTGTAGTTCG-GCAG-3′) and EX2 (5′-TGGCCGCATTCGTACAGATACGGAGG-3′); inner primer pair IN3 (5′-GCTAGAACGTATTTGCTGCAGAACG-3′) and IN4 (5′-ATCCGAAACAACTGTCTGACTGGCA-3′). (B) The products of one round of PCR amplification with primers HV7 (5′-TATCCCAGCTGT-TTTCATATAGTAAC-3′) and HV8 (5′-GCCTTGCGGTAGCACTAGATTTT-TTG-3′), specific for HHV-7 (*7*). C, negative control (no-DNA sample). M, 123-bp-ladder molecular weight markers.

uninfected and infected with HHV-6_{GS} was also included as a control. Under stringent conditions (washing with 0.5× SSC/0.1% SDS at 60°C), all the HHV-7 probes showed reactivity with HHV-7_{AL}, HHV-7_{JB}, and HHV-7_{JI} DNA, but none reacted with cellular DNA from uninfected cultures (Fig. 3), as well as from cultures infected with HHV-6, GS strain (not shown). Moreover, no hybridization with DNA from HHV-7-infected cells was observed using an HHV-6-specific probe, pZVH14 (*26*) (data not shown).

While most of the HHV-7 probes used (pVL44, pVL23, pVL18, pVL17) yielded the same restriction patterns for all three viral isolates, a genetic polymorphism was observed using one probe, pVL8 (Fig. 3). After *Hin*dIII digestion, HHV-7_{JB} and HHV-7_{JI} yielded a single large fragment of about 11 kb. In contrast, a band of lower molecular weight (approximately 5.5 kb) was seen with HHV-7_{AL} (Fig. 3A). Similarly, after *Bam*HI digestion, the restriction pattern of HHV-7_{JB} was the same as that of HHV-7_{JI}, but different from that of HHV-7_{AL} (Fig. 3B). Minimal genetic polymorphism among different HHV-7 isolates was also observed by Wyatt and Frenkel by restriction analysis of radiolabeled DNA extracted from infected cells (*9*). Whether this heterogeneity corresponds to the region recognized by our pVL8 probe remains to be defined. It may be interesting to note that the isolates displaying a similar restriction pattern were both independently derived from patients with CFS. Analysis of a wider range of isolates will permit us to verify whether the different HHV-7 strains can be classified in well-defined variants, as previously described for HHV-6 (*27–32*).

We have developed optimized conditions for the *in vitro* propagation of HHV-7. In previous reports, the virus was grown in PHA-activated, unfractionated PBMC cultures (*5, 7*) or in the neoplastic T-cell line Sup-T1 (*7*). Because the predominant target cells for HHV-7 are T lymphocytes of the $CD4^+$ subset (*5–7*), we used enriched populations of $CD4^+$ T cells derived from the peripheral blood of healthy adult blood donors. The cells were purified by negative immunomagnetic selection, performed as previously described (*8*), using magnetic beads coated with goat anti-mouse IgG antiserum (Dynal), after labeling the cells with a cocktail of MAbs to CD8 (Leu-2a), CD14 (Leu-M3), CD16 (Leu-11b), CD19 (Leu-12), and CD56 (Leu-19) (Becton Dickinson). After enrichment, the cells were stimulated with PHA (1 μg/ml) and expanded for various periods of time in the presence of IL-2. Before use, the cells were routinely screened for the presence of endogenous HHV-6 or HHV-7 by PCR and positive cultures were discarded. In enriched $CD4^+$ T cells, infection by all three HHV-7 isolates tested (JB, AL, JI) was rapid and generalized. Virus titers superior to 10^6 50% cell culture infectious doses ($CCID_{50}$)/ml were detected in the culture supernatants 7–10 days after infection, as determined by infecting triplicate cultures of normal $CD4^+$ T lymphocytes with serial 10-fold dilutions of the virus stock (i.e., cell-free supernatant from infected $CD4^+$ T cells). To evaluate the efficiency of the new culture system for HHV-7, virus titration was performed, in parallel, in purified $CD4^+$ T cells and in unfractionated PBMC (1×10^6 cells/test), both derived from the same healthy adult individual. The same HHV-7 stock yielded a $CCID_{50}$ of 10^6 in purified $CD4^+$ T cells, but only between 10^3 and 10^4 in autologous PBMC, as detected by indirect IF with MAb 9A5D12 (data not shown). Thus, the efficiency of virus expression was more than 2 logs higher in purified $CD4^+$ T cells than in unfractionated PBMC. This effect may be explained by the fact that PBMC cultures contain activated antiviral effectors, such as $CD8^+$ cytotoxic T cells and natural killer cells, that may suppress viral replication *in vitro*. A similar increase in the efficiency of infection using purified $CD4^+$ T cells was observed with the other T-lymphotropic human herpesvirus, HHV-6 (P. Lusso, unpublished observations).

As previously documented (*7*), the neoplastic $CD4^+$ T-cell line Sup-T1, originally derived from a non-Hodgkin's T-cell lymphoma (*33*), was found to be susceptible to HHV-7 infection. When inoculated with high-titered, cell-free supernatant from infected $CD4^+$ T cells, Sup-T1 cells showed generalized and progressive cytopathic effect,

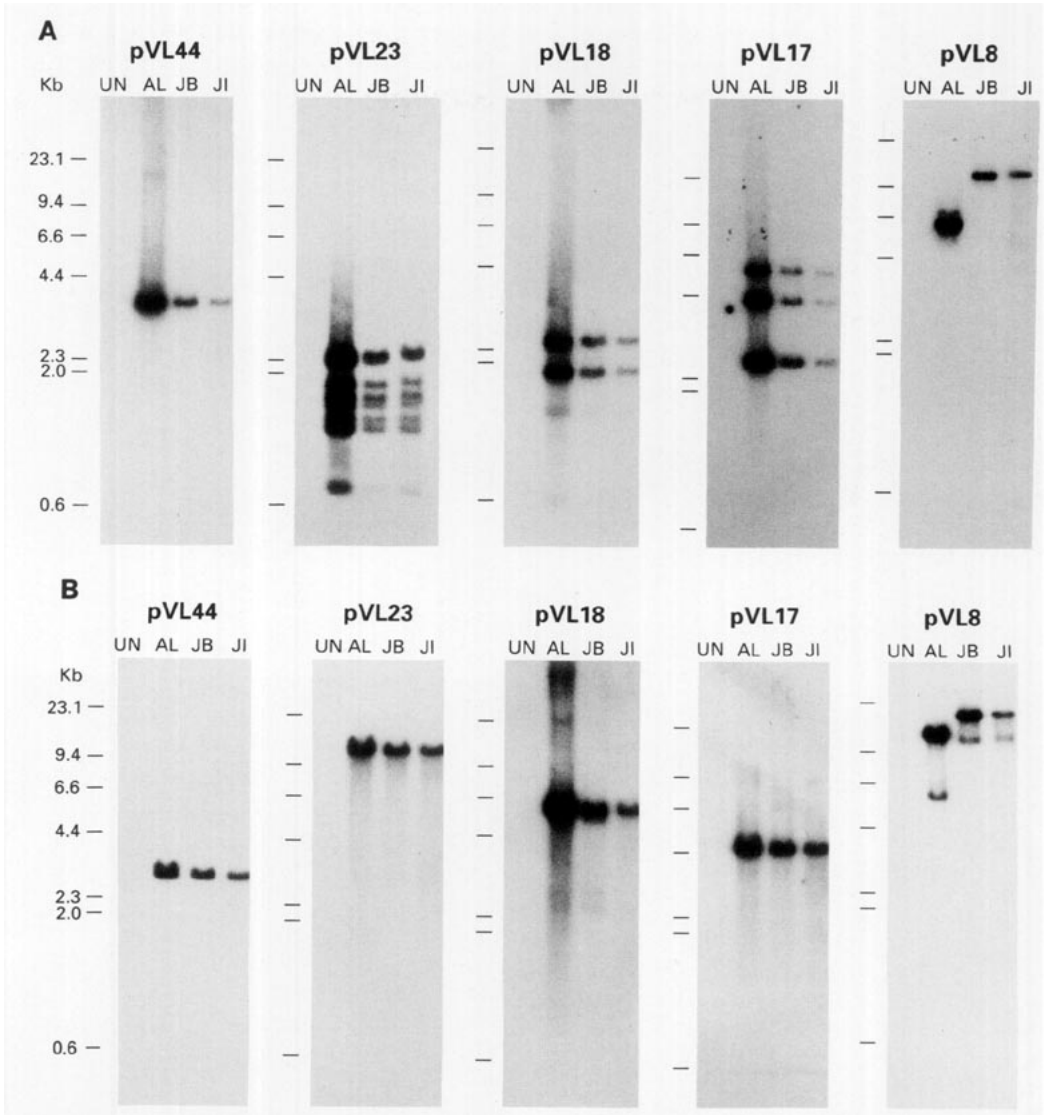

FIG. 3. Southern blot analysis of three HHV-7 isolates. DNA extracted from Sup-T1 cells infected with HHV-7_{AL}, HHV-7_{JB} and HHV-7_{JI} was digested with (A) *Hin*dIII or (B) *Bam*HI and resolved on a 0.8% agarose gel. After Southern blotting, the DNA was hybridized with different ^{32}P-labeled HHV-7-specific probes, as indicated.

with more than 80% antigen-positive cells by Day 8–10 postinfection. However, the titers of extracellular virus obtained from Sup-T1 were consistently lower (10^3–10^4 $CCID_{50}$/ml) than those obtained from purified normal $CD4^+$ T cells. Low-level HHV-7 replication was also detected in two additional neoplastic $CD4^+$ T-cell lines, Jurkat and Molt-3 (not shown).

Infection of both normal $CD4^+$ T cells and Sup-T1 cells with high-titered HHV-7 was characterized by the appearance of large, refractile cells and by an increasing loss of cell viability. A variable proportion of infected cells exhibited the typical morphological features of giant multinucleated cells, as revealed by light microscopy (Fig. 4A), electron microscopy (Fig. 4B), and nuclear IF staining with MAb 9A5D12 (Fig. 4C). The syncytia induced by HHV-7 were indistinguishable from those induced by either HHV-6 or HIV in the same cells (not shown). However, no signs of HIV infection were detected in any of the HHV-7-infected cultures (both normal $CD4^+$ T cells and Sup-T1) by PCR and p24-antigen-capture tests.

To date, no evidence of a protective humoral response to HHV-7 has been reported. We have developed a syncytia-inhibition test to detect neutralizing antibodies to HHV-7. Briefly, 2×10^5 HHV-7-infected normal $CD4^+$ T lymphocytes were pretreated for 30 min at room temperature with serial twofold dilutions of the test serum and then cocultured with 2×10^5 uninfected Sup-T1 cells in 24-well plates (Costar) in a total volume of 1 ml. Syncytia formation was scored at 20 and 40 h after cocultivation. The neutralizing antibody titer was determined as the reciprocal of the highest dilution of serum that completely prevented giant-cell formation. We determined whether

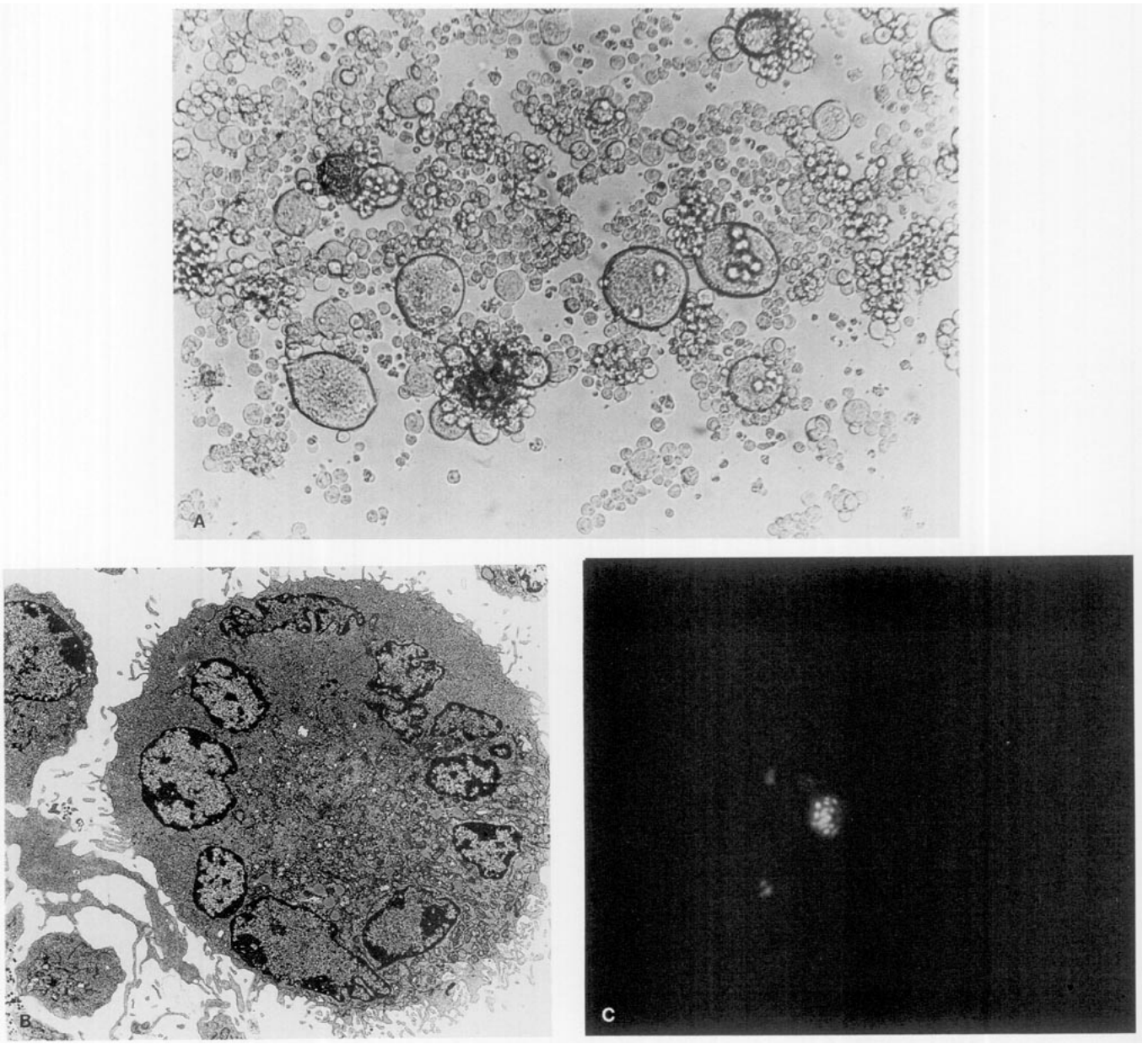

FIG. 4. Syncytia formation induced by HHV-7 infection. (A) Giant multinucleated cells in cultures of Sup-T1 cells, 5 days after infection with HHV-7_{AL}. (B) Thin sections of an infected syncytial $CD4^+$ T cell, containing several nuclei and virus particles at various stages of maturation. (C) Multinuclear IF staining with MAb 9A5D12 in HHV-7-infected peripheral blood $CD4^+$ T lymphocytes.

sera from a series of human subjects, including patients with CFS, B-cell chronic lymphocytic leukemia (CLL) and AIDS, had neutralizing antibodies to HHV-7. As seen in Table 1, all the human sera screened were found to contain syncytia-neutralizing antibodies at variable titers. A Gaussian distribution of HHV-7-neutralizing antibodies was observed in healthy adult individuals, peaking at the titer of 320. High neutralizing titers were detected in all patients with CFS, whereas patients with CLL or AIDS had low neutralizing titers. Most of these human sera were also tested in parallel for neutralization of HHV-6 syncytia formation (not shown). No direct correlation was observed between the HHV-7- and HHV-6-neutralizing antibody titers. Albeit preliminary, these data confirm the high prevalence of HHV-7 in the general population and suggest that an effective humoral response may help in controlling HHV-7 infection in immunocompetent adults.

A well-known property of herpesviruses is their ability to establish latent and persistent infection in the host (*34*). At present little is known about the ability of HHV-7 to undergo latency after primary infection. The presence of neutralizing antibodies to HHV-7, as demonstrated in all the normal human sera tested in this study, may contribute to the immunologic control of the virus.

TABLE 1

TITRATION OF SYNCYTIA-INHIBITORY ANTIBODIES TO HHV-7 IN HUMAN SERA

Patients (No. tested)	Titers of syncytia-inhibitory antibodies in serum						
	20	40	80	160	320	640	1280
AIDS (5)	1[a]	1	2	1			
CFS (5)				1	2	2	
CLL (5)	2	1	2				
Normal adults (30)	1	5	4	7	9	3	1

[a] Number of patients with the indicated syncytia-inhibitory titer. Infected human peripheral blood $CD4^+$ T cells were incubated with serial twofold dilutions of the test serum and then cocultivated with uninfected Sup-T1 cells. The titers are the reciprocal of the highest dilution that prevented giant-cell formation.

To date, no association between HHV-7 and human disease has yet emerged. The present is the second report of isolation of HHV-7 from a patient with CFS, a complex disorder of unknown etiology, in which several disturbances of the immunologic function have been documented (*35, 36*). This syndrome has been tentatively linked to diverse viral infections, including Epstein–Barr virus, HHV-6, enteroviruses and retroviruses (*35–42*). However, reactivation of silent herpesvirus infection is frequent in individuals with immunologic dysregulation and may represent a mere epiphenomenon of the underlying disease. As in the case of HHV-6, the possible association of HHV-7 with CFS is difficult to interpret due to the ubiquitous nature of these viruses and to the limited value of seroepidemiological studies (*7, 12*). Further studies are warranted to elucidate whether reinfection by or reactivation of HHV-7 might play a role in some of the clinical manifestations of CFS.

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REFERENCES

1. SALAHUDDIN, S. Z., ABLASHI, D. V., MARKHAM, P. D., JOSEPHS, S. F., STURZENEGGER, S., KAPLAN, M., HALLIGAN, G., BIBERFELD, P., WONG-STAAL, F., KRAMARSKY, B., and GALLO, R. C., *Science* **234,** 596–601 (1986).
2. DOWNING, R. G., SEWANKAMBO, N., SERWADDA, D., HONESS, R., CRAWFORD, D., JARRETT, R., and GRIFFIN, B. E., *Lancet* **2,** 390 (1987).
3. TEDDER, R. S., BRIGGS, M., CARMON, C. H., HONESS, R., ROBERTSON, D., and WHITTLE, H., *Lancet* **2,** 390–392 (1987).
4. LOPEZ, C., PELLET, P., STEWART, J., GOLDSMITH, C., SANDERLIN, K., BLACK, J., WARFIELD, D., and FEORINO, P., *J. Infect. Dis.* **157,** 1271–1273 (1988).
5. FRENKEL, N., SCHIMER, E. C., WYATT, L. S., KATSAFANAS, G., ROFFMAN, E., DANOVICH, R. M., and JUNE, C. H., *Proc. Natl. Acad. Sci. USA* **87,** 748–752 (1990).
6. BERNEMAN, Z. N., GALLO, R. C., ABLASHI, D. V., FRENKEL, N., KATSAFANAS, G., KRAMARSKY, B., and BRUS, I., *J. Infect. Dis.* **166,** 690–691 (1992).
7. BERNEMAN, Z. N., ABLASHI, D. V., LI, G., EGER-FLETCHER, M., REITZ JR., M. S., HUNG, C., BRUS, I., KOMAROFF, A. L., and GALLO, R. C., *Proc. Natl. Acad. Sci. USA* **89,** 10552–10556 (1992).
8. LUSSO, P., MARKHAM, P. D., TSCHACHLER, E., DIMARZO VERONESE, F., SALAHUDDIN, S. Z., ABLASHI, D. V., PAWHA, S., KROHN, K. J., and GALLO, R. C., *J. Exp. Med.* **167,** 1659–1670 (1988).
9. WYATT, L. S., and FRENKEL, N., *J. Virol.* **66,** 3206–3209 (1992).
10. SAXINGER, C., POLESKY, H., EBY, N., GRUFFERMAN, S., MURPHY, R., TEGTMEIR, G., PAREKH, V., MEMON, S., and HUNG, C., *J. Virol. Methods* **21,** 199–208 (1988).
11. OKUNO, T., TAKAHASHI, K., BALACHANDRA, K., SHIRAKI, K., YAMANISHI, K., TAKAHASHI, M., and BABA, K., *J. Clin. Microbiol.* **27,** 651–653 (1989).
12. WYATT, L. S., RODRIGUEZ, W. J., BALACHANDRAN, N., and FRENKEL, N., *J. Virol.* **65,** 6260–6265 (1991).
13. BRIGGS, M., FOX, J., and TEDDER, R. S., *Lancet* **1,** 1058–1059 (1988).
14. YAMANISHI, K., OKUNO, Y., SHIRAKI, K., TAKAHASHI, M., KONDO, T., ASANO, Y., and KURATA, R., *Lancet* **1,** 1065–1067 (1988).
15. CARRIGAN, D. R., DROBYSKY, W. R., RUSSLER, S. K., TAPPER, M. A., KNOX, K. K., and ASH, R. C., *Lancet* **338,** 147–149 (1991).
16. DROBYSKY, W. R., DUNNE, W. M., BURD, E. M., KNOX, K. K., ASH, R. C., HOROWITZ, M. M., FLOMENBERG, N., and CARRIGAN, D. R., *J. Infect. Dis.* **167,** 735–739 (1993).
17. CONE, R. W., HACKMAN, R. C., HUANG, M. L. W., BOWDEN, R. A., MEYERS, J. D., METCALF, M., ZEH, J., ASHLEY, R., and COREY, L., *N. Engl. J. Med.* **329,** 156–161 (1993).
18. LUSSO, P., SECCHIERO, P., CROWLEY, R. W., GARZINO-DEMO, A., BERNEMAN, Z. N., and GALLO, R. C., *Proc. Natl. Acad. Sci. USA* **91,** 3872–3876 (1994).
19. BARRE-SINOUSSI, F., CHERMANN, J. C., REY, F., NUGEYRE, M. T., CHAMARET, S., GRUEST, J., DAUGUET, C., AXLER-BLIN, C., VEZINET-BRUN, F., ROUZIOUX, C., ROZENBAUM, W., and MONTAGNIER, L., *Science* **220,** 868–871 (1983).
20. GALLO, R. C., SALAHUDDIN, S. Z., POPOVIC, M., SHEARER, G. M., KAPLAN, M., HAYNES, B. F., PALKER, T. J., REDFIELD, R., OLESKE, J., SAFAI, B., WHITE, G., FORESTER, P., and MARKHAM, P., *Science* **224,** 500–503 (1984).
21. DALGLEISH, A. G., BEVERLY, P., CLAPHAM, P., CRAWFORD, D., GREAVES, M. F., and WEISS, R. A., *Nature* **312,** 763–766 (1984).
22. KLATZMAN, D., CHAMPAGNE, E., CHARMAREF, S., GRUEST, J., GUETARD, D., HERCEND, T., GLUCKMAN, J. C., and MONTAGNIER, L., *Nature* **312,** 767–768 (1984).
23. ROIZMAN, B., *In* "The Herpesviruses" (B. Roizman, Ed.), pp. 1–23. Plenum, New York, 1982.
24. BALACHANDRAN, N., AMELSE, R. E., ZHOU, W. W., and CHANG, C. K., *J. Virol.* **63,** 2835–2840 (1989).
25. KLOTMAN, M. E., LUSSO, P., BACCHUS, D., CORBELLINO, M., JARRETT, R. F., and BERNEMAN, Z. N., *In* "Diagnostic Molecular Microbiology" (D. H. Persing, Ed.), pp. 501–510. American Society for Microbiology, Washington, DC, 1993.
26. JOSEPHS, S. F., SALAHUDDIN, S. Z., ABLASHI, D. V., SCHACHTER, F., WONG-STAAL, F., and GALLO, R. C., *Science* **234,** 601–603 (1986).
27. ABLASHI, D. V., BALACHANDRAN, N., JOSEPHS, S. F., HUNG, C. L., KRUEGER, G. R. F., KRAMARSKY, B., SALAHUDDIN, S. Z., and GALLO, R. C., *Virology* **184,** 545–552 (1991).
28. AUBIN, J. T., COLLANDRE, H., CANDOTTI, D., INGRAND, D., ROUZIOUX, C., BURGARD, M., RICHARD, S., HURAUX, J. M., and AGUT, H., *J. Clin. Microbiol.* **29,** 367–372 (1991).
29. SCHIRMER, E. C., WYATT, L. S., YAMANISHI, K., RODRIGUEZ, W. J., and FRENKEL, N., *Proc. Natl. Acad. Sci. USA* **88,** 5922–5926 (1991).
30. ABLASHI, D., AGUT, H., BERNEMAN, Z., CAMPADELLI-FIUME, G., CARRIGAN, D., CECCERINI-NELLI, L., CHANDRAN, B., CHOU, S., COLLANDRE, H., CONE, R., DAMBAUGH, T., DEWHURST, S., DI LUCA, D., FOA-

TOMASI, L., FLECKENSTEIN, B., FRENKEL, N., GALLO, R., GOMPELS, U., HALL, C., JONES, M., LAWRENCE, G., MARTIN, M., MONTGNIER, L., NEIPEL, F., NICHOLAS, J., PELLETT, P., RAZZAQUE, A., TORRELLI, G., THOMSON, B., SALAHUDDIN, S., WYATT, L., and YAMANISHI, K., *Arch. Virol.* **129,** 365–366 (1993).

31. AUBIN, J.-T., AGUT, H., COLLANDRE, H., YAMANISHI, K., CHANDRAN, B., MONTAGNIER, L., and HURAUX, J.-M., *J. Virol. Methods* **41,** 223–234 (1993).

32. GOMPELS, U. A., CARRIGAN, D. R., CARSS, A. L., and ARNO, J., *J. Gen. Virol.* **74,** 613–622 (1993).

33. SMITH, S. D., SHATSKY, M., COHEN, P. S., WARNKE, R., LINK, M. P., and GLADER, B. E., *Cancer Res.* **44,** 5657–5660 (1984).

34. STEVENS, J. G., *Microbiol. Rev.* **53,** 318–332 (1989).

35. BUCHWALD, D., CHENEY, P. R., PETERSON, D. L., HENRY, B., WORMSLEY, S. B., GEIGER, A., ABLASHI, D. V., SALAHUDDIN, S. Z., SAXINGER, C., BIDDLE, R., KIKINIS, R., JOLESZ, F. A., FOLKS, T., BALACHANDRAN, N., PETER, J. B., GALLO, R. C., and KOMAROFF, A. L., *Ann. Intern. Med.* **116,** 103–113 (1992).

36. KOMAROFF, A. L., GUPTA, S., and SALIT, I. E., *In* "Human Herpesvirus-6" (D. V. Ablashi, G. R. F. Krueger, and S. Z. Salahuddin, Eds.), pp. 235–253. Elsevier, Amsterdam, 1992.

37. ABLASHI, D. V., JOSEPHS, S. F., BUCHBINDER, A., HELLMAN, K., NAKAMURA, S., LLANA, T., LUSSO, P., KAPLAN, M., DAHLBERG, J., MEMON, S., IMAM, F., ABLASHI, K. L., MARKHAM, P. D., KRAMARSKY, B., KRUEGER, G. R. F., BIBERFELD, P., WONG-STAAL, F., SALAHUDDIN, S. Z., and GALLO, R. C., *J. Virol. Methods* **21,** 29–48 (1988).

38. ARCHARD, L. C., BOWLES, N. E., BEHAN, P. O., BELL, E. J., and DOYLE, D., *J. R. Soc. Med.* **81,** 326–329 (1988).

39. DALE, J. K., STRAUSS, S. E., ABLASHI, D. V., SALAHUDDIN, Z. S., GALLO, R. C., NISHIBE, Y., and INOUE, Y. K., *Ann. Intern. Med.* **110,** 92–93 (1989).

40. DE FREITAS, E., HILLIARD, B., CHENEV, P. R., BELL, D. S., KIGGUNDU, E., SANKEY, D., WROBLEWSKA, Z., PALLADINO, M., WOODWARD, J. P., and KOPROWSKI, H., *Proc. Natl. Acad. Sci. USA* **88,** 2922–2926 (1991).

41. JONES, J. F., and STRAUSS, S. E., *Annu. Rev. Med.* **38,** 195–209 (1987).

42. KRUEGER, G. R. F., KOCH, B., and ABLASHI, D. V., *Lancet* **2,** 36 (1987).