JOURNAL OF CLINICAL MICROBIOLOGY, June 1995, p. 1660–1661 Vol. 33, No. 6
0095-1137/95/$04.00+0

Human Herpesvirus 6 and Human Herpesvirus 7 in Chronic Fatigue Syndrome

DARIO DI LUCA,[1] MARCELLA ZORZENON,[2] PRISCO MIRANDOLA,[1] ROBERTO COLLE,[3] GIUSEPPE A. BOTTA,[2] AND ENZO CASSAI[1]*

Institute of Microbiology, University of Ferrara, Ferrara,[1] and Institute of Microbiology, University of Udine,[2] and 2nd Medical Department, Civil Hospital,[3] Udine, Italy

Received 14 November 1994/Returned for modification 27 January 1995/Accepted 6 March 1995

We analyzed lymphocytes of patients with chronic fatigue syndrome (CFS) for the presence of human herpesvirus 6 (HHV-6) and HHV-7 DNA. HHV-7 was present in over 80% of CFS patients and healthy controls, while the prevalence of HHV-6 variant A increased significantly in CFS cases (22 versus 4%; $P = 0.05$).

Chronic fatigue syndrome (CFS) is a vaguely defined illness characterized by debilitating fatigue and weakness lasting for more than 6 months. The cause of CFS is still unknown, and a working case definition has recently been proposed (7). Viruses, including human herpesvirus 6 (HHV-6), have been suspected to be triggering agents for CFS. In some studies CFS patients had HHV-6 antibody titers higher than those of controls (5), but in other studies there were no differences between patients and controls (8). These results are difficult to interpret for several reasons: (i) HHV-6 infection is highly prevalent in the adult population; (ii) some reagents employed may have cross-reacted with the closely related HHV-7; (iii) CFS patients have higher than average antibody titers for other viruses also. One study described active HHV-6 replication in several CFS patients (4), but some objections about the study design and the experimental procedures were raised (12). Nothing is known of a possible association of HHV-7 with CFS.

We have screened peripheral blood mononuclear cells from healthy donors and CFS patients for the presence of HHV-6 and HHV-7 DNA by PCR. Thirty-six patients who fulfilled the CFS criteria (7) and 24 unselected healthy blood donors matched for age and sex from the same geographical area were enrolled in the study. All patients were afflicted by a constellation of symptoms, including moderate fever lasting more than 6 months, indolent lymphoadenopathy, excessive muscle fatigue on previously well-tolerated mild exercise, myalgias, and short-term memory disturbance. None of the patients had laboratory or clinical signs of active hepatitis, and all of the patients had negative serology for Lyme disease. Enterovirus infection was excluded by fecal cultures. No evidence of autoimmune disorders was obtained, and psychiatric disorders were ruled out by a specialist.

Peripheral blood mononuclear cells were purified on Ficoll gradients, and DNA was extracted by conventional procedures. PCR testing was done on 1 μg of DNA (corresponding to 1.5×10^5 diploid cells) with primers specific for HHV-6 and HHV-7. The primers for HHV-6 were developed by Aubin et al. (1), and the primers for HHV-7 were described by Berneman et al. (2). Particular care was taken to avoid contamination of PCR samples. Several blank reactions with no or unrelated DNA were interspersed with the samples, the positive controls were always handled separately from the samples, and three different rooms, each with its own reagents and equipment, were used to set up the reactions.

The amplification products were analyzed by Southern hybridization with specific, radiolabelled oligonucleotide probes. Experiments of sensitivity, run in parallel, showed that the equivalent of 20 DNA molecules in 150,000 cells was consistently detected (6) (data not shown). The hybridization results are shown in Table 1. HHV-7 had the same prevalence in healthy donors and CFS patients, being detected in 83 and 82% of the samples, respectively. HHV-6 was detected in 29% of the samples from healthy individuals and in 44% of the samples from CFS patients.

HHV-6 strains are divided into two variants that differ in biologic, molecular, and immunologic properties and seem to be differentially distributed in the healthy population, variant B being more frequent than variant A (6). Characterization of HHV-6 variants was performed by analysis of variant-specific restriction sites. Variant B was similarly distributed between healthy and CFS samples. Interestingly, the prevalence of variant A was higher in CFS patients, reaching the same frequency as variant B (Table 1).

To our knowledge, this is the first description showing the high prevalence of HHV-7 in peripheral blood mononuclear cells and the similar distribution of the virus in healthy individuals and CFS patients. Considering the high detection frequency of HHV-7, no conclusions about possible associations with CFS can be drawn.

The overall prevalence of HHV-6 in healthy individuals is similar to that some of us recently described in the Italian population (6). It should be noted that higher frequencies of detection (up to 90%) were obtained due to the analysis of more cells (9) than in our study, showing that, as for HHV-7, infection and persistence of HHV-6 are very common but, in contrast to HHV-7, HHV-6 sequences appear to be less abundant in peripheral blood. Interestingly, the distribution of HHV-6 variants in CFS patients seems to differ from that in healthy controls. In fact, while the levels of variant B remain unaltered, the prevalence of HHV-6 variant A (HHV-6A) increases significantly ($P = 0.05$; two-tailed Fisher's exact test).

An earlier study described reactivation of HHV-6 in three of seven CFS patients (10); the viral variants were not determined in that study, but from the restriction pattern it is possible to infer that all three isolates belonged to variant A. The present findings substantiate that observation, and the P value obtained, within the limits of statistical significance, hints at the importance of confirming these results with samples of larger size. At the moment, it is not possible to determine whether

* Corresponding author. Mailing address: Institute of Microbiology, University of Ferrara, Via Borsari 46, 44100 Ferrara, Italy. Phone: (39) 532 291407. Fax: (39) 532 247618.

TABLE 1. Prevalence of HHV-6 and HHV-7 in CFS

Peripheral blood lymphocytes from:	No. with virus/no. tested (%)			
	HHV-7	HHV-6		
		Variant B	Variant A	Total
Healthy donors	20/24 (83)	6/24 (25)	1/24 (4)	7/24 (29)
CFS patients	23/28 (82)	8/36 (22)	8/36 (22)	16/36 (44)

HHV-6A reactivation or infection could be etiologically associated with at least some cases of CFS or might be just an epiphenomenon reflecting immune dysregulation (11). However, CFS is the second example of a human disease in which the prevalence of HHV-6A is increased. The previous description of increased HHV-6A prevalence was in Kaposi's sarcoma, and in that case it was also postulated that HHV-6 variants could exhibit differential reactivation rates (3). In any event, HHV-6A and HHV-6B seem to behave differently in specific diseases.

This work was supported by the Ministero della Sanità 1994 AIDS Project, AIRC, ACRO (to E. Cassai), and CNR (to G. A. Botta).

We thank G. Pascuttini for technical assistance.

REFERENCES

1. **Aubin, J. T., H. Collandre, D. Candotti, D. Ingrand, C. Rouzioux, M. Burgard, S. Richard, J. M. Huraux, and H. Agut.** 1991. Several groups among human herpesvirus 6 strains can be distinguished by Southern blotting and polymerase chain reaction. J. Clin. Microbiol. **29:**367–372.
2. **Berneman, Z. N., D. V. Ablashi, G. Li, M. Eger-Fletcher, M. S. Reitz, 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. **Bovenzi, P., P. Mirandola, P. Secchiero, R. Strumia, E. Cassai, and D. Di Luca.** 1993. Human herpesvirus 6 (variant A) in Kaposi's sarcoma. Lancet **341:**1288–1289.
4. **Buchwald, D., P. R. Cheney, D. L. Peterson, B. Henry, S. B. Wormsley, A. Geiger, D. V. Ablashi, S. Z. Salahuddin, C. Saxinger, R. Biddle, R. Kikinis, F. A. Jolesz, T. Folks, N. Balachandran, J. B. Peter, R. C. Gallo, and A. L. Komaroff.** 1992. A chronic illness characterized by fatigue, neurologic and immunologic disorders, and active HHV-6 infection. Ann. Intern. Med. **116:**103–113.
5. **Dale, J. K., S. E. Strauss, D. V. Ablashi, S. Z. Salahuddin, R. C. Gallo, Y. Nishibe, and I. K. Inoue.** 1989. The Inoue-Melnick virus, human herpesvirus type 6, and the chronic fatigue syndrome. Ann. Intern. Med. **110:**92–93.
6. **Di Luca, D., R. Dolcetti, P. Mirandola, V. De Re, P. Secchiero, A. Carbone, M. Boiocchi, and E. Cassai.** 1994. Human herpesvirus 6: a survey of presence and variant distribution in normal peripheral lymphocytes and lymphoproliferative disorders. J. Infect. Dis. **170:**211–215.
7. **Fukuda, K., S. E. Straus, I. Hickie, M. C. Sharpe, J. G. Dobbins, A. Komaroff, A. Schluederberg, J. F. Jones, A. R. Lloyd, S. Wessely, N. M. Gantz, G. P. Holmes, D. Buchwald, S. Abbey, J. Rest, J. A. Levy, H. Jolson, D. L. Peterson, J. H. Vercoulen, U. Tirelli, B. Evengard, B. H. Natelson, L. Steele, M. Reyes, and W. C. Reeves.** 1994. The chronic fatigue syndrome—a comprehensive approach to its definition and study. Ann. Intern. Med. **121:**953–959.
8. **Gold, D., R. Bowden, J. Sixbey, R. Riggs, W. J. Katon, R. Ashley, R. Obrigewitch, and L. Corey.** 1990. Chronic fatigue. A prospective clinical and virologic study. JAMA **264:**48–53.
9. **Jarrett, R. F., D. A. Clark, S. F. Josephs, and D. E. Onions.** 1990. Detection of human herpesvirus 6 DNA in peripheral blood and saliva. J. Med. Virol. **32:**73–76.
10. **Josephs, S. F., B. Henry, N. Balachandran, D. Strayer, D. Peterson, A. L. Komaroff, and D. V. Ablashi.** 1991. HHV-6 reactivation in chronic fatigue syndrome. Lancet **337:**1346–1347.
11. **Komaroff, A. L., S. Gupta, and I. E. Salit.** 1992. Post-viral chronic fatigue syndrome, p. 235–253. *In* D. V. Ablashi, G. R. F. Krueger, and S. Z. Salahuddin (ed.), Human herpesvirus 6. Elsevier, Amsterdam.
12. **Reeves, W. C., P. E. Pellett, and H. Gary.** 1992. The chronic fatigue syndrome controversy. Ann. Intern. Med. **117:**343.