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Use of anti HHV-6 transfer factor for the treatment of two patients with Chronic Fatigue Syndrome (CFS). Two case reports

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Abstract

Specific Human Herpes virus-6 (HHV-6) transfer factor (TF) preparation, administered to two chronic fatigue syndrome patients, inhibited the HHV-6 infection. Prior to treatment, both patients exhibited an activated HHV-6 infection. TF treatment significantly improved the clinical manifestations of CFS in one patient who resumed normal duties within weeks, whereas no clinical improvement was observed in the second patient. It is concluded that HHV-6 specific TF may be of significant value in controlling HHV-6 infection and related illnesses.

Abbreviations: CFS : chronic fatigue syndrome; HCBMC : human cord blood mononuclear cells; HHV-6 : human herpes virus type 6; PBL : peripheral blood lymphocytes; PBMC : cultured peripheral blood mononuclear cells; TF : transfer factor.

Introduction

HHV-6 was first isolated in 1986 from patients with AIDS and lymphoproliferative disorders [1]. Since then, HHV-6 has been shown to be a common infectious agent in the general population and could be isolated from healthy donors, or patients with immune deficiencies, auto-immune diseases, lymphoproliferative disorders and chronic fatigue syndrome (CFS)[2]. Several RNA and DNA viruses have been implicated in CFS, including HHV-6, but thus far none of them have been found to be the causative agent [3,4]. However, higher reactivation of HHV-6 has been reported by various investigators [5–11], suggesting that this virus may play an important role in the pathogenesis of the syndrome. These observations are based on significantly elevated IgG antibody titers to HHV-6 (>3–4 fold increase), detection of IgM antibody in some patients who showed antigen-producing cells in their cultured peripheral blood mononuclear cells (PBMC), detection of HHV-6 DNA in the PBMC and significantly elevated IgG and IgM antibody titers to HHV-6 early protein P41/38 [4].

Based on preliminary in vivo and in vitro studies[12–15], several antiviral agents have been suggested for CFS therapy, but, thus far, there is no accepted specific treatment for this syndrome. Due to lack of toxicity, transfer factor (TF) has been effectively used in a number of viral diseases in previous studies [16–21]. Because of the high reactivation rate of HHV-6 in CFS patients, lack of specific treatment, and absence of TF toxicity in vivo, we were tempted to evaluate the anti-HHV-6 activity of TF on certain CFS patients with HHV-6 active infection. The HHV-6 specific TF was prepared from immune murine lymphocytes and subsequently replicated in the LDV/7 cell line [22,23]. It was orally administered to two CFS patients.

Materials and methods

Patients

Patient 1, a 24-year-old male student, became ill in June 1992 with severe fatigue, muscular pain, and sleep disorders. He consulted an infectious disease specialist in November 1992, who found elevated CMV IgG antibodies and up to 25% atypical lymphocytes. There was also an increase in his IgA, which progressively increased to 605 mg/dl. In August 1992, the patient had a 25% increase in NK cells with absence of clonality and lymphocyte gene rearrangement. He developed acute anxiety problems with apparent depression and mental confusion. He also exhibited other symptoms associated with CFS. Tests for Lyme disease were found negative. In December 1994, his PBMC cells were cultured for HHV-6 and two months later a second blood sample was drawn and processed for HHV-6 infection. At this time, his serum was tested for antibodies to HHV-6 and to EBV. After finding an active HHV-6 infection, HHV-6 specific TF therapy was initiated in December 1994, TF was orally administered.

Patient 2, a 27-year-old female student, developed in June 1991 fatigue with sore throat, tender swollen lymphnodes, headaches, difficulty in concentrating, memory loss, depression, and a number of other symptoms compatible with CFS diagnosis, including night sweats, seizures, and anxiety attacks. She was forced to discontinue her schooling and on March 1992 was treated orally with HHV-6 specific transfer factor.

Transfer factor

BALB/c mice were injected SQ with a preparation containing HHV-6, EBV, and CMV live viruses. The mice were sacrificed 3–4 weeks later and TF was obtained from their spleen cells. It was in vitro replicated using the LDV/7 cells, a B-lymphoblastoid cell line which contains EBV-DNA, but lacks virus production, as previously described [22]. TF was given orally in capsule form. Patients were given 2 capsules every third day, i.e., 10^8 cell equivalent.

Detection of HHV-6. (A) Culture of PBMC.

PBMC from the 2 patients, after lymphocyte separation ($\leq 1 \times 10^6$/ml), were propagated in the presence of PHA (5mg/ml). From patient 1 the PBMC, after 48 hours mitogen stimulation, were centrifuged and then were either cultured in RPMI 1640 medium containing 10% fetal bovine serum and 10% IL-2 (Advanced Biotechnologies Inc, Columbia, MD), or they were co-cultivated with PHA-stimulated human cord blood mononuclear cells (HCBMC) in the presence of IL-2 and cell culture medium. The cell cultures were incubated at 37°C in presence of 5% $C0_2$, and were examined (a) by light microscopy for morphological changes, i.e., formation of typical enlarged cells, (b) by monoclonal antibodies to HHV-6 early (P41/38) or late (gp116/64/54) antigens by indirect immunofluorescence assay (IFA) at 3,7,14 days post incubation. If the cells were found to be positive for late antigens, the supernatant from the infected culture (antigen positive) was filtered through 0.45μ filter and inoculated into PHA-stimulated HCBMC for cell-free infection. The infected cells were examined by electron microscopy and the virus isolate characterized as either HHV-6 Variant A or Variant B [24]. The protocol for the detection of HHV-6 in PBLs from patient 2 was similar to patient 1 .

(B) Detection of antibody to HHV-6

During separation of the patients' PBL, the plasma was saved and titered for IgG antibody using IFA [13,14]. Plasma was also assayed for presence of IgM antibody by IFA. For detection of IgM, plasma was incubated with the substrate, i.e. HHV-6 infected H582 cells for a period of 3 hours, after adsorption with Gull absorbent to remove IgG antibody. After the 3 hour incubation period, the cells were washed in PBS and incubated with rabbit anti-human IgM FITC for 35 minutes. The cells were then washed, counterstained with Evans blue (1:700 dilution) mounted and examined for fluorescent staining.

(C) Detection of HHV-6 capsid antigen by antigen capture assay

The plasma, as well as the cell culture fluid, from the PBMC of the patient 1 were tested by the HHV-6 antigen capture ELISA to gp116 (Advanced Biotechnologies Inc, Columbia, MD), according to the procedure described in the kit.

Table 1. Detection of HHV-6 Infection in Peripheral Blood Mononuclear Cells (PBMC) from Patient 1*

Sample I.D.		Detection HHV-6 Antigen in cultured cells				Detection of HHV-6 Antigen (core protein) by Antigen Capture Assay	
		PBMC		PBMC Co-cultivated with cord blood mononuclear cells		Antigen in Tissue culture fluid from cells	Antigen in Plasma
		Early (p41/38)	Late (gp116)	Early(p41/38)	Late (gp116)		
HCBMC Dec. 12, 1994	Day 3	>5%	<3%	>10%	±15%	+	+
	Day 7	>10%	>15%	<20%	>25%	+	No sample
	*Day 14	<20%	<25%	>30%	>35%	+	No sample
HCBMC Feb. 17, 1995	Day 3	–	–	–	–	–	–
	Day 7	–	–	–	–	–	No sample
	Day 14	–	–	–	–	–	No sample

*Cell-free supernatant was used to infect (a) PHA-stimulated HCBMC, (b) J-Jhan and MoLT-3 T-cell lines. HCBMC became HHV-6 positive by Day 3 and only J-Jhan cell line showed positivity to HHV-6 by Day 7 post infection, suggesting that HHV-6 isolate from Patient 1 is Variant A [20].

Results

(A)HHV-6. Profile of patient 1

Table 1 shows that patient 1 had active HHV-6 infection in December 1994, when his PBMC were cultured. The antigen-expressing cells were detected with HHV-6 Mabs as early as post-culture day 3. The antigen-expressing cells increased in number, especially the balloon-type large cells, as the culture grew older (Fig. 1). Table 1 shows that the patient's plasma obtained in December 1994 also exhibited the presence of HHV-6 antigen. PBMC whether cultured by themselves or co-cultivated with HCBMC showed the presence of cell-free virus, since these samples were positive for HHV-6 antigen by the antigen capture ELISA, and cell-free supernatant was able to infect fresh HCBMC by Day 3. The HCBMC infected cells under electron microscopy showed typical herpes virus particles (Fig. 2). Moreover, the PBMC culture showed some large balloon-shaped cells which were found in clusters or appeared singly. Morphologically, these cells were identical to the HHV-6 infected cells, previously reported in the literature [1].

Our data also show that the isolate of HHV-6 recovered from patient 1 is Variant A type, since it grew in H5B2 and J-Jhan cells, but not in the MoLT-3 cells [24]. Moreover, J-Jhan infected cells did not react with OHV-3 (P98) monoclonal antibody to HHV- -6, which only reacts with Variant B types. This isolate also gave a restriction enzyme pattern of Variant A [24].

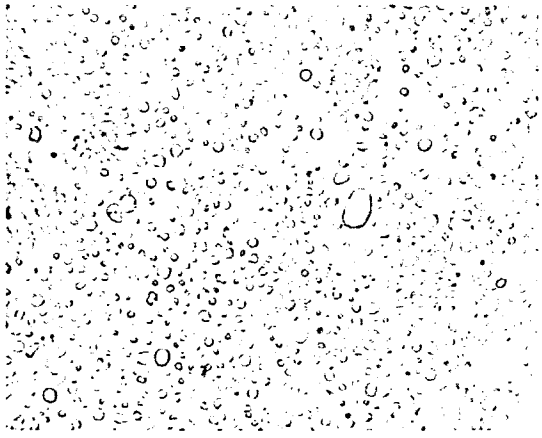

Figure 1. In vitro culture of PBMC from patients 1 showing enlarged cells after 14 days post culture (1). Clumps of PBMC are visible in the culture (Darker). Some of the cells in the culture contained medium to large HHV-6 antigen positive cells when tested with Mabs to HHV-6.

Table 2 shows the HHV-6 antibody titers of patient 1. At the time of the first culture of PBMC (December, 1994), the plasma sample showed significantly elevated IgG antibody titers, as tested by IFA. The same sample of plasma contained IgM antibody; however, the second sample of serum obtained in January 1995 showed a drop in IgG antibody and no IgM antibody was detectable. A plasma sample, obtained in February 1995, showed a considerable drop in IgG antibody.

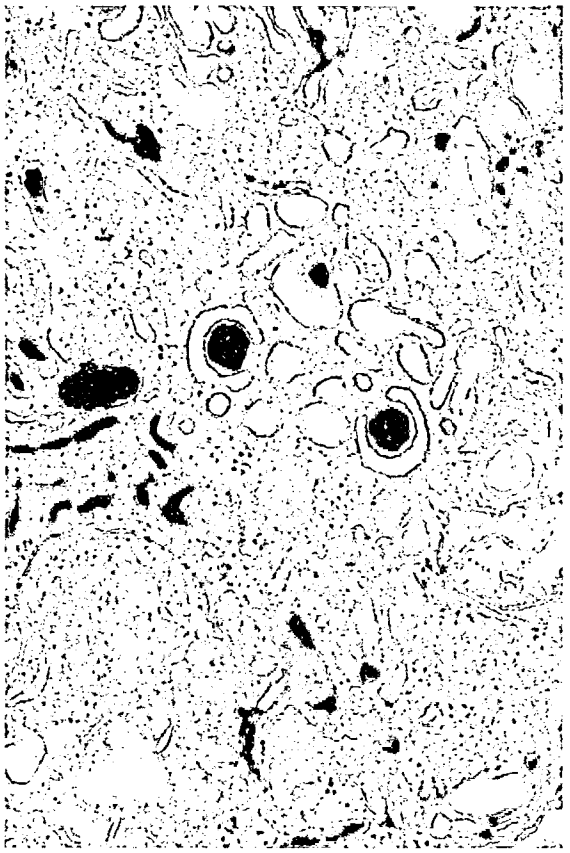

Figure 2. An electron micrograph of HHV-6 viral particles from PBMC co- cultivated with HCBMC from patient 1. The two mature virions typical of a herpes virus (1) are shown on the micrograph.

Table 2. HHV-6 Antibody Profile of Patient 1

Sample I.D.	IgG	IgM
Dec. 1994 Plasma*	1:256	$\leq$1:20
Jan. 1995 Serum	1:640	Negative at 1:10
Feb. 1995 Plasma*	$\leq$1:160	Negative at 1:10

*Plasma was obtained from the same samples of peripheral blood which were used for establishing a cell culture. The same plasma samples were used for the HHV-6 antigen capture assay as shown in Table 1.

The presence of HHV-6 IgM antibody and elevated IgG antibody are highly suggestive of virus reactivation. The antibody profile data showed a correlation with the detection of HHV-6 antigens in the patient's peripheral blood lymphocytes (PBL)(Table 1). Thus, Tables 1 and 2 suggest that the patient had an active HHV-6 infection which subsided over a two month period, perhaps due to the HHV-6-specific TF administration.

(B) HHV-6. Profile of patient 2

Prior to TF therapy, the PBMC from patient 2 were cultured as described for patient 1. Fourteen days post culture, a significant number of enlarged HHV-6 antigen positive cells were observed in the culture as shown in Figure 1 for patient 1. HHV-6 could be recovered from the supernatant of the PBMC.

Patient 2 received TF from March 1992 to August 1993. Prior to TF treatment, her EBV IgG antibody titer for VCA was 1:2560; it declined during the TF treatment to 1:1280 and then to 1:640. Her EBV-EA IgG titer was 1:10–1:320; after TF administration it decreased to 1:20–1:80 and then to <1:10. This patient was EBV IgM antibody negative (<1:10) and EBNA antibody positive.

Patient 2's HHV-6 IgG antibody titer, prior to TF therapy was between 1:2560–1:5120; after TF treatment it dropped to >1:20–1:320 and $\leq$1:320, whereas 5 months after TF therapy, her PBMC, upon culture, failed to exhibit any HHV-6-antigen positive cells, even when the cells were co-cultivated.

Effect of TF on patients' clinical condition.

Patient 1 did not show any clinical improvement of his CFS symptoms, even though there was no sign of active HHV-6 infection after two months of TF therapy. However, the second patient continued to improve physically and eventually she resumed normal activities. Her condition, two years later, is absolutely normal.

Discussion

The causative agents of CFS are not yet identified. However, it is probable that active chronic viral infection, such as EBV and/or HHV-6, could play a role in the etiopathology of the syndrome. But, besides the viral agents, other factors could share a responsibility in the pathogenesis of CFS. Whatever factors were responsible for the onset of the CFS symptomatology in patient 2, the virus-specific TF seems to have suppressed their effect, together with the presence of HHV-6 and EBV infection, thereby improving the patient's clinical condition.

Patient 1 did not show clinical improvement, the CFS symptomatology remained unaltered, despite control of the HHV-6 infection. Indeed, while he was actively replicating HHV-6 before viral specific TF

therapy, i.e., at the time his first PBMC sample was cultured (December 12, 1994) (Table 1), the second PBMC, taken one month after the first sample of PBMC was drawn (January 14, 1995), showed absence of HHV-6 infected cells. No virus could be detected by IFA or antigen capture assay in the PBMC sample taken approximately one month after the initiation of TF therapy (February 17, 1995), suggesting that HHV-6 had been suppressed or cleared from the host. It is plausible to speculate that this was due to the effect of the HHV-6-specific TF on HHV-6 replication.

Additional evidence for active HHV-6 infection in this patient was offered by the presence of IgM antibody and significantly elevated IgG antibody to HHV-6. HHV-6 IgM antibody, like cytomegalovirus IgM antibody, correlates with active virus replication in vivo. Two serum and plasma samples drawn later show that no IgM antibody to HHV-6 was detectable, while IgG antibody showed a drop from 1:2560 to 1:160. These data further support the hypothesis that HHV-6-specific TF is capable of inhibiting HHV-6 infection in vivo.

Patient 2 showed a similar pattern. Her EBV-VCA antibody titers dropped from 1:2560 to 1:640 and her EBV-EA titers eventually dropped below detectable levels. The HHV-6 antigen and antibody profiles showed that no HHV-6 could be recovered after treatment with virus-specific TF. Also her IgG antibody dropped from 1:5120 to $\leq$1:320, suggesting that TF suppressed both HHV-6 and EBV replication.

The inhibitory effect of HHV-6-specific TF warrants further clinical studies. It is plausible that its clinical usefulness in CFS patients will be proven in larger clinical studies, although, if CFS is a multicausal syndrome, the immediate clinical benefit may not be the same in all patients, the control of two viral infections may not be sufficient to tip the balance and reverse the course of the syndrome.

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References

1. Salahuddin SZ, Ablashi DV, Markham PD, Josephs SF, Sturzenegger S, Kaplan M, Haligan G, Biberfeld P, Wong-Staal F, Kramarsky B & Gallo Re. Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders, Science 1986; 234: 596–601.
2. Krueger GRF, Kluepeiberg U & Hoffmann A. Raman A. Clinical correlates of infection with human herpesvirus-6. In Vivo 1994; 8: 457–86.
3. Ablashi DV: Viral studies of chronic fatigue syndrome. Clin Inf D 1994; 18: 5130–133.
4. Ablashi DV, Ablashi KL, Kramarsky B, Bernbaum JB, Whitman JW, & Pearson GR. Viruses and chronic fatigue syndrome: Current status. J Chronic Fatigue Syndrome: 1995; 2: 3–22.
5. Buchwald D, Cheney PR, Peterson DL, Henry B, Wormsley SB, Geiger A, Ablashi DV, Salahuddin SZ, Saxinger C, Biddle R, Kilkinis R, Jolesz FA, Folks T, Balachandran N, Gallo RD & Komaroff AL. A chronic illness characterized by fatigue, neuralgia, and immunologic disorders and active herpesvirus-6 type infection. Ann Int Med 1992; 116: 103–13.
6. Joseph SF, Henry B, Balachandran N, Strayer D, Peterson D, Komaroff AL & Ablashi DV. HHV-6 reactivation in chronic fatigue syndrome. Lancet 1991; 337: 1346–47.
7. Hilgers A, Krueger GRF & Lembke H. A post infection chronic fatigue syndrome case history of 35 patients in Germany. In Vivo 1991; 5: 201–06.
8. Yalcin S, Kuratsune H, Yamaguchi K, Kitani T & Yaminishi K. Prevalence of human herpesvirus-6 Variants A and B in patients with chronic fatigue syndrome. Microb Immu 1994; 38: 587– 90.
9. Zorzenon N, Colle R, Rukh G, Barsanti LA, Botta GA & Ceccherini-Nelli L. Active HHV-6 infection in chronic fatigue syndrome patients from Italy: new data. J Chronic Fatigue Syndrome: 1996; 2: 3–12.
10. Ablashi DV, Zompetta C, Lease C, Josephs SF, Balachandran N, Kamaroff AL, Krueger GRF, Henry B, Luka J & Salahuddin SZ. Human herpesvirus-6 (HHV-6) and chronic fatigue syndrome (CFS). Can Dis Weekly Report 1991; 175E: 33–40.
11. Levine PH, Jacobson S, Pocinke AG, Cheney P, Peterson D, Connelly RR, Weil R, Robinson SM, Ablashi DV, Salahuddin SZ, Pearson GR & Hoover R. Clinical epidemiologic and virologic studies in four clusters of chronic fatigue syndrome. Arch In Med 1992; 152: 1611–16.
12. Williams M: HHV-6: response to antiviral agents. In: Ablashi DV, Krueger GRF, Salahuddin SZ. eds. Human Herpesvirus-6. Amsterdam: Elsevier Science Publisher, B.V, 1992: 317–35.
13. Ablashi DV, Berneman ZN, Lawyer C, Kramarsky B, Ferguson DM & Komaroff AL. Antiviral activity in vitro of Kutapressin against human herpesvirus-6. In Vivo 1994; 8: 581–86.
14. Ablashi DV, Berneman ZN, Williams M, Strayer DR & Komaroff AL. Ampligen inhibit human herpesvirus-6. In Vivo 1994; 8: 587–92.
15. Strayer DR, Carter W, Strauss KL, Brodsky L, Suhadolnik RJ, Ablashi DV, Henry B, Mitchel WN, Bastien L & Peterson D. Long-term improvements in patients with chronic fatigue syndrome treated with ampligen. J Chronic Fatigue Syndrome 1994; 1: 35–53.
16. Neequaye J, Viza D, Pizza G, Levine PH, De Vinci C, Ablashi DV, Biggar RJ & Nkrumah FK. Specific transfer factor with activity against Epstein-Barr virus reduces late relapse in endemic Burkitt Lymphoma. Anticancer Res. 1990, 10: 1183–88.

17. Viza D, Vich JM, Phillips J & Rosenfeld F. Orally administered specific transfer factor for the treatment of herpes infections. Lymphok Res 1985; 4: 27–30.
18. Roda E, Viza D, Pizza G, Mastroroberto L, Phillips J, De Vinci C & Barbara L. Transfer factor for the treatment of HBsAg-positive chronic active hepatitis. P Soc Exp Med 1985; 178: 468–75.
19. Nkrumah F, Pizza G, Viza D, Phillips J, DeVinci C & Levine P. Regression of progressive lymphadenopathy in a young child with acute cytomegalovirus (CMV) infection following the administration of transfer factor with specific anti-CMV activity. Lymphok Res 1985; 4: 237–41.
20. Pizza G, Viza D, De Vinci C, Palareti A, Cuzzocrea D, Fornarola V & Baricordi R. Orally administered HSV- specific transfer factor (TF) prevents genital and/or labial herpes relapses. Biotherapy: this issue.
21. Prasad U, Amin Jalaludin MA, Rajadurai P, Pizza G, De Vinci C, Viza D & Levine P. Transfer Tactor with anti-EBV activity as an adjuvant therapy for nasopharyngeal carcinoma: a pilot study. Biotherapy: this issue.
22. Viza D, Goust JM, Moulias R, Trejdosiewicz LK, Collard A & Müller-Bérat N. 'In vitro' production of transfer factor by lymphoblastoid cell lines. Transplan P 1975; VII (suppl 1): 329–33.
23. Viza D, Boucheix CI, Césarini JP, Ablashi DV, Armstrong G, Levine P & Pizza G. Characterization of a human lymphoblastoid cell line, LDV/7, used to replicate transfer factor and immune RNA. Bio Cell 1982; 46: 1–10.
24. Ablashi DV, Agut H & Berneman ZN et al. Human herpesvirus-6 strain groups nomenclature. Arch. Viral 1993, 129:363–66.

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