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Human Herpesvirus 6 and 7 Reactivation and Disease Activity in Multiple Sclerosis

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Key words: *multiple sclerosis; human herpesvirus 6; human herpesvirus 7; plasma viremia; interleukin 12; tumor necrosis factor α.*

Summary. *Recent studies have focused on the associations between human herpesvirus 6 (HHV-6) and human herpesvirus 7 (HHV-7), and multiple sclerosis (MS).*

The aim of this study was to investigate the associations between HHV-6 and HHV-7 reactivation and MS disease activity, and interleukin 12 (IL-12) and tumor necrosis factor α (TNF-α) production.

Material and Methods. The frequency of plasma viremia by nested polymerase chain reaction and transcription of viral mRNA in peripheral blood mononuclear cells by reverse transcriptase-polymerase chain reaction (RT-PCR) of 14 relapsing/remitting (RR) and 14 secondary progressive (SP) MS patients were studied in comparison with clinical manifestation of the disease. Serum concentrations of cytokines IL-12 and TNF-α were analyzed by enzyme-linked immunosorbent assay.

Results. Plasma samples from 25 of the 28 MS patients with estimated latent/persistent HHV-6 and/or HHV-7 infection were examined during relapse and remission/relative remission. HHV-6 reactivation was found in 4 of the 7 RRMS and 4 of the 7 SPMS patients, and HHV-7 reactivation was identified in 3 of the 7 RRMS and 1 of the 7 SPMS patients (all in relapse). In 2 of the 3 RRMS patients without viremia in relapse, HHV-6 mRNA transcription was detected. In RRMS and SPMS patients with active HHV-6 and HHV-7 infection in relapse, the serum concentrations of IL-12 and TNF-α were significantly higher than in those with latent virus infection.

Conclusions. HHV-6 and HHV-7 reactivation could be implicated in the exacerbation of MS via activation of Th1 lymphocyte subsets.

Introduction

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system, the etiology of which is thought to have a possible viral component. Several immunological and molecular studies have supported a relationship between human herpesvirus 6 (HHV-6) and MS (1–11). Moreover, the associations between HHV-6 reactivation and MS disease activity have been observed, suggesting that HHV-6 reactivation is implicated in the exacerbation of MS (11–13). The balance between proinflammatory and anti-inflammatory cytokines has been shown to be associated with the disease activity in MS (14). Interleukin 12 (IL-12) is an immunoregulatory cytokine with a broad range of activities including the regulation of cytokine synthesis and selective promotion of T-lymphocyte subpopulation of T-helper type 1 (Th1) development. Th1-type cells producing disease-promoting proinflammatory cytokines, one of which is tumor necrosis factor α (TNF-α), were found to be a key factor in the onset of autoimmune processes in MS (15, 16).

Our previously studies have shown the association of HHV-6 with MS and correlation between HHV-6 reactivation and MS disease activity (17, 18). Although active HHV-7 infection was found more frequently in MS patients than control blood donors, its possible involvement in demyelinating processes has to be elucidated (17, 19). HHV-7 is an enveloped, double-stranded DNA beta-herpesvirus that is closely related to HHV-6. Like other herpesviruses, HHV-7 becomes latent after primary infection and can be reactivated (20). This study examined the relationship between the reactivation of both closely related human herpesviruses HHV-6 and HHV-7 and disease activity in relapsing/remitting (RR) and secondary progressive (SP) MS. Plasma viremia and transcription of viral mRNA in PBMCs were used as the markers of HHV-6 and HHV-7 active infection, and they correlated with clinical and MRI evidence of MS activity as well as

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with serum IL-12 and TNF-α concentrations. The aim of this study was to evaluate the associations between HHV-6 and HHV-7 reactivation and MS disease activity, and IL-12 and TNF-α production.

Material and Methods

Patients. Twenty-eight randomly selected patients with MS (21 females and 7 males) were examined for evidence of HHV-6 and HHV-7 infection. The mean age of the patients was 37 years (range, 16–59 years). The cohorts were established with the approval of the local Ethics Committee, and all the participants gave informed consent before the examination. A clinical diagnosis of MS was established according to the criteria of McDonald et al. (21) and confirmed by brain MRI. The mean duration of disease was 7 years (range, 1–22 years). Fourteen patients had relapsing-remitting MS (RRMS) and 14 secondary progressive MS (SPMS). All patients had not received immunosuppressive drugs within 3 months before the study.

Brain Magnetic Resonance Imagining. Magnetic resonance imaging (MRI) was carried out according to the standard protocol using a 1.5-T system. The patients were considered to have active inflammatory lesions (positive gadolinium [Gd]-enhanced lesions) when the areas of hyperintensity, compared to surrounding brain parenchyma, were recorded on a T1-weighted MRI scan after intravenous injection of Gd-DTPA (0.1 mmol/kg body weight).

Detection of HHV-6 and HHV-7 Genomic Sequences by Polymerase Chain Reaction. Total DNA was extracted from peripheral blood mononuclear cells (PBMCs) and cell-free plasma. Nested polymerase chain reaction (nPCR) was used for the detection of HHV-6 and HHV-7 genomic sequences. Detection of HHV-6 was carried out according to Secchiero et al. (22) and HHV-7 according to Berneman et al. (23). In our experiments, the sensitivity of HHV-6-specific primers corresponded to 3 copies of the HHV-6 genome and the sensitivity of HHV-7-specific primers to 5 copies of the HHV-7 genome (17).

Detection of HHV-6 and HHV-7 mRNA Transcription by Reverse Transcriptase-Polymerase Chain Reaction. Total RNA was extracted from 5×10^6 PBMCs by the guanidinium isothiocyanate-phenol method using TRI Reagent™ LS (Sigma, USA) according to the manufacturer's protocol. Reverse transcription was carried out in a 20-μL volume according to the manufacturer's protocol (Thermo Fisher Scientific, Lithuania). The samples were then subjected to amplification with the same primers for HHV-6 or HHV-7 as used in the nPCR.

Enzyme-linked immunosorbent assay. A commercial enzyme-linked immunosorbent assay (ELISA) kit (Bender MedSystem, Austria) was used to measure the concentration of IL-12 (p70) and TNF-α in serum samples according to the manufacturer's protocol. Kallikrein (Sigma, USA) (1000 IE/mL) was added to the serum samples to prevent protein degradation.

Statistical Analysis. The serum concentrations of IL-12 and TNF-α are expressed as mean (SD). The Student *t* test was used to compare continuous variables with a *P* value of <0.05 considered as significant.

Results

Of the 28 MS patients, 25 had latent/persistent HHV-6 or/and HHV-7 infection. HHV-6 sequence in PBMC DNA was found in 9 (64.3%) of the 14 patients with RRMS and 9 (64.3%) of the 14 with SPMS; HHV-7 sequence was identified in 10 (71.4%) of the 14 patients with RRMS and 13 (92.9%) of the 14 with SPMS. In 7 (58.3%) of the 12 patients with RRMS and 9 (69.2%) of the 13 with SPMS, double infection was detected.

Relationship Between HHV-6 and HHV-7 Reactivation and Disease Activity in Multiple Sclerosis. Cell-free plasma samples from 25 MS patients with latent/persistent HHV-6 and/or HHV-7 infections were examined during the periods of relapse and remission/relative remission. HHV-6 genomic DNA in plasma was detected in 4 of the 7 RRMS and 4 of the 7 SPMS patients examined during the exacerbation period confirmed by the presence of Gd-enhanced lesions on MRI, but not during the period of remission or relative/remission when HHV-6 infection remained latent and Gd-enhanced lesions were absent (Table 1). The transcription of viral mRNA was detected in 2 of the 3 PBMC RNA samples from RRMS patients in an exacerbation phase confirmed by brain MRI, but without HHV-6 plasma viremia. HHV-7 plasma viremia was found in 3 of the 7 RRMS and in 1 of the 7 SPMS patients all being in the period of clinical exacerbation confirmed by the Gd-enhanced lesions on MRI, but not in any of the patients during the period of clinical remission/relative remission and absence of Gd-enhanced lesions on MRI (Table 1). No transcription of HHV-7 mRNA was found in PBMC RNA from 4 RRMS and 6 SPMS patients despite the active phase of the disease and Gd-enhanced lesions on MRI (Table 1). Thus, the reactivation of HHV-6 (10/14; 71.4%) and HHV-7 (4/14; 28.6%) was detected in MS patients only during the exacerbation phase in both RRMS and SPMS. Two patients with single HHV-7 latent/persistent infection were examined repeatedly in the periods of exacerbation and remission/relative remission (Table 2). Active HHV-7 infection was detected only during the clinical exacerbation and MRI positive for Gd-enhanced lesions in both RRMS and SPMS (Table 2). In two RRMS patients with dual HHV-6 and -7 infection in the active phase of the disease confirmed by brain MRI, the reactivation of HHV-7 but not HHV-6 was detected.

Table 1. Relationship Between HHV-6 and HHV-7 Reactivation and Disease Activity in Multiple Sclerosis

Markers of Active Viral Infection and Disease Activity	Type and Phase of Multiple Sclerosis			
	Relapsing/Remitting		Secondary Progressive	
	Exacerbation	Remission	Exacerbation	Relative Remission
HHV-6 DNA in plasma	4/7	0/2	4/7	0/2
HHV-6 mRNA in PBMCs	2/3	0/2	0/3	0/2
MRI	7/7	0/2	7/7	0/2
HHV-7 DNA in plasma	3/7	0/3	1/7	0/6
HHV-7 mRNA in PBMCs	0/4	0/3	0/6	0/6
MRI	7/7	0/3	7/7	0/6

Values are number of positive cases/number of tested cases.
PBMCs, peripheral blood mononuclear cells; MRI, magnetic resonance imaging; HHV-6, human herpesvirus 6; HHV-7, human herpesvirus 7.

Table 2. Associations Between Activity of HHV-7 Infection and Activities of RRMS and SPMS During Examination of Two Patients in Dynamics

Patients	Activity Phase of the Disease	HHV-7 in Plasma (nPCR)	MRI Gd+	IL-12, pg/mL (ELISA)	TNF-α, pg/mL (ELISA)
RRMS	Exacerbation	+	+	21.6	128.4
	Exacerbation	+	+	21.2	65.1
	Remission	–	–	<5	12.7
SPMS	Exacerbation	+	+	17.3	136.0
	Exacerbation	+	+	21.0	270.0
	Relative remission	–	–	<5	24.2
	Exacerbation	+	+	22.0	147.0

IL-12, interleukin 12; TNF-α, tumor necrosis factor α; HHV-6, human herpesvirus 6; HHV-7, human herpesvirus 7; MRI, magnetic resonance imaging; ELISA, enzyme-linked immunosorbent assay; RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis.

Table 3. Associations Between Serum IL-12 and TNF-α Levels and Activity of HHV-6 and HHV-7 Infections During Different Types and Phases of Multiple Sclerosis

Marker and Infection Type		Virus	Type and Phase of Multiple Sclerosis			
			Relapsing/Remitting		Secondary Progressive	
			Exacerbation	Remission	Exacerbation	Relative Remission
IL-12, pg/mL	Active infection	HHV-6	24.70 (4.10)	–	22.28 (1.89)	–
		HHV-7	20.62 (0.96)	–	20.10 (1.43)	–
	Latent infection	HHV-6	9.10 (3.11)	<5	9.97 (1.16)	7.80 (3.91)
		HHV-7	9.10 (3.11)	<5	12.04 (1.73)	5.97 (2.67)
TNF-α, pg/mL	Active infection	HHV-6	169.95 (44.98)	–	106.75 (15.28)	–
		HHV-7	111.40 (30.37)	–	184.33 (43.00)	–
	Latent infection	HHV-6	22.30 (1.00)	17.22 (9.23)	51.93 (12.25)	52.30 (17.91)
		HHV-7	22.30 (1.00)	18.96 (8.90)	43.58 (6.91)	57.95 (21.50)

Values are given as mean (SD). IL-12, interleukin 12; TNF-α, tumor necrosis factor α; HHV-6, human herpesvirus 6; HHV-7, human herpesvirus 7.

Influence of HHV-6 and HHV-7 Activation on Serum Levels of IL-12 and TNF-α. In RRMS and SPMS, the mean serum levels of IL-12 in the disease exacerbation phase and active HHV-6 infection were significantly higher in comparison with the levels in the exacerbation phase with latent HHV-6 infection ($P<0.05$ and $P<0.01$, respectively) (Table 3). Similarly, the mean serum concentration of TNF-α in the period of RRMS relapse and active HHV-6 infection was significantly higher compared with that in the periods of relapse and remission when HHV-6 infection was latent ($P<0.05$ and $P<0.025$, respectively). A significant difference was found also between mean serum TNF-α levels during SPMS relapse and active HHV-6 infection, relapse and latent HHV-6 infection, and relative remission ($P<0.05$) (Table 3).

The mean serum concentrations of IL-12 and TNF-α were significantly higher among the patients in RRMS relapse and with active HHV-7 infection

in comparison with the concentrations of these interleukins in RRMS patients in relapse and latent HHV-7 infection ($P<0.05$) (Table 3). In SPMS patients in the exacerbation phase with active HHV-7 infection, the mean serum concentrations of IL-12 and TNF-α were significantly higher compared with the concentrations of these interleukins in the periods of disease activity and relative remission when HHV-7 infection was latent ($P<0.05$) (Table 3).

High serum levels of IL-12 (23.6 pg/mL and 18.3 pg/mL, respectively) and TNF-α (190.1 pg/mL and 62.0 pg/mL, respectively) were detected in two RRMS patients in relapse with HHV-7 reactivation from the background of latent/persistent double infection. Similar data were obtained in the RRMS and SPMS patients with single HHV-7 infection: significantly higher serum levels of IL-12 and TNF-α in the period of relapse and HHV-7 activation in comparison with these indices in the remission period and latent HHV-7 infection were documented ($P<0.05$) (Table 2). Thus, an obvious association between HHV-6 and HHV-7 reactivation and serum concentrations of IL-12 and TNF-α was demonstrated.

Discussion

MS is usually diagnosed in the second or third decade of life, and it is difficult to prove a causative association with HHV-6 and HHV-7 infections, which in the event of acute infection during childhood does not usually produce acute after-effects (2). Recently, much attention has been given to the relationship between HHV-6 and MS, but there are only few studies regarding the significance of HHV-7 in the etiopathogenesis of MS. HHV-6 and HHV-7 are closely related viruses and have very similar biological behavior. Both viruses infect cells of the immune system and thus may modulate their function (24, 25). Since HHV-6 latently infects CD4+ lymphocytes and low-level expression (chronic infection) occurs, HHV-6 protein is represented in the cell membrane, and due to this, T lymphocytes start to treat these cells as foreign. This HHV-6 protein present in the cell membrane has a 10-amino acid homology with myelin basic protein (MBP), so the organism also starts to recognize MBP as foreign, which is the basis of the autoimmune process (25). In our previously studies, the association of HHV-6 with MS and correlation between HHV-6 reactivation and MS disease activity were reported (17, 18). Many researchers consider MS as a CD4+ Th1-mediated inflammatory demyelinating disease (26, 27), in the pathogenesis of which, the variety of cytokines are involved either in the induction phase or the effector phase. IL-12 is a cytokine thought to play a major role in the pathogenesis of MS (28); TNF-α is a critical cytokine in the effector phase. Our study aimed to establish whether a relationship existed between HHV-6 and HHV-7 reactivation and MS disease activity, and IL-12 and TNF-α production. HHV-6 as well as HHV-7 reactivation is demonstrated in RRMS and SPMS patients only during the relapse phase of the disease confirmed by the presence of Gd-enhanced lesions in the brain. Two patients repeatedly in the periods of disease exacerbation and remission/relative remission were examined, and it has been shown for the first time that active HHV-7 infection was detected only during the clinical exacerbation in RRMS and SPMS.

The fact that HHV-6 and HHV-7 genome sequences are not found in the plasma of some RRMS and SPMS patients during flare-ups could be related to the early stage of virus replication when the virus has not lysed the cells yet. This is confirmed by the detection of HHV-6 mRNA transcription in two RRMS patients during a flare-up in spite of the fact that the presence of the virus genome sequence in DNA plasma samples was not found. Moreover, patients with active HHV-6 or HHV-7 infection in relapse demonstrate significantly higher concentrations of proinflammatory cytokines IL-12 and TNF-α in peripheral blood in comparison with patients with latent virus infection in relapse. The recent studies on the function of some herpesvirus genome products and their interaction with the host immune system have suggested that the reactivation of HHV-6 and HHV-7 may act as initiators in the cascade of reactions in the immune system causing a relapse of MS (14, 29). Our studies demonstrated the correlation between HHV-6 and HHV-7 reactivation and disease activity in both RRMS and SPMS. Simultaneous virus reactivation and an increase in the concentrations of cytokines IL-12 and TNF-α indicate that these viruses could be implicated in MS exacerbation via activation and proliferation of Th1 lymphocyte subset. Active HHV-6 as well as HHV-7 infection may play at least a cofactor role in the initiation and progression of the MS disease.

Conclusions

HHV-6 and HHV-7 reactivation could be implicated in the exacerbation of multiple sclerosis via activation of Th1 lymphocyte subsets.

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Statement of Conflict of Interest

The authors state no conflict of interest.

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