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Correlation Between HHV-6 Reactivation and Multiple Sclerosis Disease Activity

Svetlana Chapenko,[1]* Andrejs Millers,[2] Zaiga Nora,[1] Inara Logina,[2] Rita Kukaine,[1] and Modra Murovska[1]

[1]*August Kirchenstein Institute of Microbiology and Virology, University of Latvia, Riga, Latvia*
[2]*Latvian Medical Academy, Riga, Latvia*

This study examined the association between HHV-6 infection and multiple sclerosis (MS) and the relationship between HHV-6 reactivation and disease activity. The frequency of HHV-6 genomic sequences in peripheral blood mononuclear cells (PBMCs), the incidence of plasma viremia (nPCR), the transcription of viral mRNA in PBMCs (RT-PCR), the presence of antiviral IgM and IgG class antibodies in the plasma (IFA) of 16 relapsing/remitting and secondary progressive MS patients were studied in comparison with clinical manifestations of the disease, magnetic resonance imaging (MRI) of brain, and serum interleukin (IL)-12 concentrations (ELISA). The prevalence of HHV-6 infection was significantly higher in patients with MS (16/26) than in patients with other neurological diseases (6/21) and in blood donors (43/150). HHV-6 reactivation was found during periods of disease activity with Gadolinium-enhancing lesions on MRI in both relapsing/remitting and secondary progressive MS (10/13; 76.9%). In patients with active MS disease, serum concentrations of IL-12 were significantly higher in those patients with active HHV-6 infection than in patients with latent infection. The data confirm an association between HHV-6 infection and MS and show a correlation between HHV-6 reactivation and disease activity in relapsing/remitting and secondary progressive MS. The risk of an exacerbation of MS was significantly higher ($P < 0.005$) in patients with active HHV-6 infection than in patients with latent infection. A clear correlation between HHV-6 reactivation and serum IL-12 concentrations during disease activity has been demonstrated. The results suggest that HHV-6 reactivation is implicated in exacerbation of MS, possibly through modulation of IL-12 synthesis. ***J. Med. Virol. 69:111–117, 2003.*** © 2003 Wiley-Liss, Inc.

KEY WORDS: latent infection; viremia; mRNA transcription; serum/plasma antibodies; IL-12; MRI

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS). It is generally accepted that MS is a systemic T-cell-mediated autoimmune disease [Martino and Hartung, 1999] with a T-helper type 1 (Th1) profile of cytokine production [Carrieri et al., 1992]; however, the precise etiology of MS remains unknown. Viruses have long been proposed to be either initiating factors for or directly pathogenic in the development of MS [Cermelli and Jacobson, 2000]. The balance between pro-inflammatory and anti-inflammatory cytokines has been shown to be associated with the disease activity in MS [van Boxel-Desaire et al., 1999]. Interleukin-12 (IL-12) is an immunoregulatory cytokine with a broad range of activities including regulation of cytokine synthesis and selective promotion of Th1-type cell development [Trinchieri, 1994; Zhang and Kaplan, 2000]. Magnetic resonance imaging (MRI) of the brain is important in the diagnosis of MS and in monitoring disease activity and evolution [Filippi, 1998; Weiner et al., 2000].

During the last few years much attention has been paid to the study of the association between human herpesvirus 6 (HHV-6) infection and MS. HHV-6, first isolated in 1986 [Salahuddin et al., 1986], is classified as a member of the order *Caudovirales*, family *Herpesviridae*, subfamily *Betaherpesvirinae* and genus *Roseolovirus* [Levy, 1997] and is further classified into two variants, HHV-6A and HHV-6B [Ablashi et al., 1993]. The primary infection, which occurs in early childhood, is followed by lifelong latency/persistency. In contrast with an earlier view of HHV-6 as a predominantly T-lymphotropic virus, infection has been found in early

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*Correspondence to: Svetlana Chapenko, August Kirchenstein Institute of Microbiology and Virology, University of Latvia, Ratsupites St. 1, Riga, Latvia, LV-1067. E-mail: modra@latnet.lv

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bone marrow progenitor cells, NK cells, monocytes/macrophages and B-lymphocytes [Campadelli-Fiume et al., 1999]. The virus productively infects CD4+, CD8+ T-cells, monocytes/macrophages, epithelial and dendritic cells [Campadelli-Fiume et al., 1999]. HHV-6 infects various cell types of the CNS in vitro [He et al., 1996; Albright et al., 1998] and in vivo [Challoner et al., 1995; Friedman et al., 1999; Knox et al., 2000] including astrocytes, oligodendrocytes and microglia. It has been suggested that the virus invades the CNS during primary infection [Yoshikawa et al., 2000] and that the CNS is a site of virus persistence [Chan et al., 1999].

An association between HHV-6 and MS has been suggested by: 1) the demonstration of viral antigen expression in the nuclei of oligodendrocytes in inflammatory lesions of brain tissue [Challoner et al., 1995]; 2) the detection of IgM and increased IgG specific anti-HHV-6 antibody titers in plasma/serum [Soldan et al., 1997; Ablashi et al., 1998, 2000] and in cerebrospinal fluid (CSF) [Ablashi et al., 1998; Ongradi et al., 1999]; and 3) the detection of HHV-6 DNA in peripheral blood mononuclear cells (PBMCs) [Ablashi et al., 2000; Alvarez-Lafuente et al., 2002], brain tissue [Challoner et al., 1995; Friedman et al., 1999], blood plasma/serum and CSF [Soldan et al., 1997; Ablashi et al., 1998] of patients with MS, but not in controls. In other studies, however, evidence for the association between HHV-6 and MS has not been found although a pathogenic role in some MS patients was not excluded [Campadelli-Fiume et al., 1999; Hay and Tenser, 2000; Taus et al., 2000]. Therefore, the role of HHV-6 in the pathogenesis of MS remains controversial.

We have studied the relationship between HHV-6 reactivation and disease activity in two types of MS, relapsing/remitting and secondary progressive. Plasma viremia, the detection of the viral mRNA transcription in PBMCs, and the presence of IgM or an increased titer of anti-HHV-6 IgG antibodies in patients with latent/persistent HHV-6 infection and MS were correlated with clinical and MRI evidence of disease activity and with serum IL-12 concentrations.

MATERIALS AND METHODS

Patients and Controls

Twenty-six patients with MS (20 females, 6 males), 21 patients (13 females, 8 males) with other neurological disorders (multisystem atrophy, motor neuron disease, stroke, inherited ataxias, Parkinson disease) and 150 randomly selected blood donors (77 females, 73 males) as controls were investigated for evidence of HHV-6 infection. The mean age of the patients with MS was 34 years (16–57 years), of the patients with other neurological disorders, 37 years (21–62), and of the blood donors, 37 years (18–60). 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 according to the criteria of Poser et al. [1983] was confirmed by brain MRI. The mean duration of disease was 6 years (1–22 years). Eleven patients had relapsing/remitting MS and 15 had secondary progressive MS. The patients were further characterized by the presence or absence of disease activity. Active disease was defined as a clinical exacerbation and the presence of active inflammatory lesions on MRI; quiescent (inactive) disease was defined as a clinical remission in the relapsing/remitting form and as relative stability in the secondary progressive form and in both forms required an absence of active inflammatory lesions on MRI.

Sixteen of 26 patients with MS (7 relapsing/remitting, 9 secondary progressive) were positive for HHV-6 infection and underwent further examination. For the seven relapsing/remitting patients the HHV-6 studies were undertaken during an exacerbation in three, during both an exacerbation and a remission in three and only during a remission in one. Of nine patients with secondary progressive MS seven were examined during a period of clinical deterioration (disease activity) and two during relative stability (relative remission). The patients did not receive immunosuppressive drugs for 3 months before the study.

Brain MRI

MRI was carried out according to the standard protocol outlined by Miller et al. [1991] using a 1.5-T system. The patients were considered to have acute inflammatory lesions (positive Gadolinium [Gd]-enhancing lesions) when areas of hyperintensity, compared to surrounding brain parenchyma, were recorded on a T1-weighted MRI scan after i.v. injection of Gd-DTPA (0.1 mmol/kg b.wt.).

Blood Sampling

EDTA blood was collected by venepuncture. Plasma samples were aliquoted and stored at −70°C. Blood samples from patients with MS were taken on the same day as the Gd-enhanced MRI.

PBMC Isolation

PBMCs were separated by Ficoll-Hypaque (Pharmacia Biotech AB, Sweden) density gradient centrifugation and stored at −70°C.

Detection of HHV-6 Genomic Sequences by Polymerase Chain Reaction (PCR)

Total DNA was extracted from PBMCs and blood plasma. The cells were lysed in 80 μl of 5× proteinase K buffer (0.375 M NaCl, 12 M EDTA pH 8.0), 20 μl SDS, 30 μl of proteinase K (10 mg/ml) and 240 μl of deionized water (to final volume 400 μl), followed by incubation at 55°C for 1 hr. DNA was extracted by phenol-chloroform treatment, precipitated by ethanol, dissolved in 50–150 μl deionized water and stored at −70°C. The QIAamp Blood Kit (Qiagen GmbH, Germany) was used for the extraction of DNA from 200 μl blood plasma

samples according to the manufacturer's instructions. A globin PCR was carried out to assure the quality of the extracted DNA [Vandamme et al., 1995].

Nested PCR (nPCR) was used for the detection of HHV-6 sequences in PBMC DNA and blood plasma DNA from patients with MS and other neurological disorders, and from control blood donors. Each PCR contained 1 μg of DNA in a final volume of 50 μl with PCR buffer (Fermentas, Lithuania), 0.2 mM of each deoxyribonucleoside triphosphate, 1.5 mM $MgCl_2$, 1U Taq DNA polymerase (Fermentas, Lithuania) and 0.2 μM of each specific primer. DNA isolated from HHV-6B (Z-29) virions (donated by Dr. S. Dewhurst, University of Rochester Medical Center, NY) was used as the positive control. To exclude the possibility of contamination during the PCR, HHV-6 negative DNA, as well as water controls, were included in each experiment.

The detection of HHV-6 DNA was carried out according to Bandobashi et al. [1997] with a nested primer set, complementary to the gene coding the major capsid protein, which recognizes both variants (A and B) of the virus. The nucleotide sequence of the primers 5′-GCGTTTTCAGTGTGTAGTTCGGCAG-3′ and 5′-TGGCCGCATTCGTACAGATACGGAGG-3′(outer pairs); 5′-GCTAGAACGTATTTGCTGCAGAACG-3′ and 5′-ATCCGAAACAACTGTCTGACTGGCA-3′ (inner pairs) for the first and second round of PCR, respectively. The inner primers amplify a 258-bp fragment of HHV-6. The first round of PCR was carried out at 94°C for 3 min, followed by 30 cycles of 94°C for 1 min, 57°C for 1 min, and 72°C for 1 min. Terminal extension of 72°C for 5 min was carried out after completion of the 30 cycles. A sample (10 μl) of the first round product was used as template for the second round using the conditions described for the first round. The products of DNA amplification were analyzed by electrophoresis on a 1.7% agarose gel and visualized under UV with ethidium bromide staining.

Detection of HHV-6 mRNA Transcription by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from 5×10^6 PBMCs by guanidinium isothiocyanate phenol method using TRI Reagent™ LS (Sigma, St. Louis, MO) according to the manufacturer's protocol. The quality of the RNA preparations was controlled by amplifying a 650-bp fragment of the β-actin gene [Noppen et al., 1997]. In addition, the absence of residual genomic DNA in the mRNA preparation was tested by carrying out PCR before cDNA synthesis without any positive results.

Reverse transcription was carried out in 20 μl volume according to the manufacturer's protocol (MBI Fermentas, Lithuania). The reaction mixture contained RT buffer (50 mM Tris-HCl pH 8.3 at 25°C, 50 mM KCl, 4 mM $MgCl_2$, 10 mM DTT), 200 ng of RNA, specific primers (20 pmol/μl), 10 mM 4-dNTP mix (1 mM final concentration), ribonuclease inhibitor (1 U/μl), and M-MuLV (Moloney murine leukemia virus) reverse transcriptase (2 U/μl). After reverse transcription at 37°C for 1.5 hr the reaction was stopped by heating to 70°C for 10 min and then chilling on ice. The samples were then subjected to amplification with the same primers for HHV-6 as used in the nPCR described above. PCR and RT-PCR were carried out using reagents of MBI Fermentas (Lithuania).

Indirect Immunofluorescence Assay (IFA)

IIFA kits (EUROIMMUN, Germany) were used for the detection of IgM and IgG antibodies to HHV-6 in plasma. Before determination of IgM specific antibodies IgG class antibodies were removed from the patient's plasma by immunoabsorption (EUROSORB: IgG/RF Absorbent) according to the manufacturer's protocol (EUROIMMUN, Germany). A titer of 1:10 or higher was considered positive.

ELISA

A commercial ELISA (Bender MedSystem, Austria) was used to measure the concentration of IL-12 (p70) in serum samples according to the manufacturer's protocol. Kallikrein (Sigma, St. Louis, MO) (1000 IE/ml) was added to the serum samples to prevent protein degradation. The assay was able to detect 5 pg/ml IL-12. The sera of seven relapsing/remitting MS and nine secondary progressive MS patients in different disease phases (20 samples in total) and of six patients with other neurological disorders (6 samples) were studied.

Statistical Methods

Statistical difference in the prevalence of HHV-6 infection between tested groups was assessed by the Fisher's exact test. The serum concentration of IL-12 was expressed as mean ± SD. Student's *t*-test was used to assess the analysis of continuous variable values with value of $P < 0.05$ considered as significant. Relative risk was calculated using the Word Processing, Database, and Statistic Program for Public Health Epi Info 6, Version 6.04B [Centers for Disease Control and Prevention (CDC), USA, World Health Organization, Geneva, Switzerland].

RESULTS

Prevalence of HHV-6 Infection Among Patients With MS, Patients With Other Neurological Disorders, and Blood Donors

HHV-6 genomic sequences were found in 16/26 (61.5%) PBMC DNA samples of patients with MS, in 6/21 (28.6%) patients with other neurological disorders and in 43/150 (28.7%) blood donors (Table I). The difference in prevalence of HHV-6 between patients with MS and patients with other neurological disease ($P = 0.039$) and between patients with MS and blood donors ($P = 0.0027$) was statistically significant. HHV-6 DNA in blood plasma was detected in 8/16 (50.0%) patients with MS but not in any of the plasma samples of patients

TABLE I. Detection of HHV-6 Infection by nPCR*

Groups	Patients (*n*)	HHV-6 DNA[a]	
		PBMC *n* (%)	Plasma[b] *n* (%)
MS (total)	26	16 (61.5)	8 (50.0)
Relapsing/remitting MS	11	7 (63.6)	4 (57.0)
Secondary progressive MS	15	9 (60.0)	4 (44.0)
Other neurological disorders	21	6 (28.6)	0 (0.0)
Blood donors	150	43 (28.7)	0 (0.0)

*nPCR, nested polymerase chain reaction; MS, multiple sclerosis; PBMC, peripheral blood mononuclear cells.
[a]Number of positives and % positives of number tested.
[b]Plasma of patients positive for HHV-6 in PBMC DNA were tested.

with other neurological disease or of the blood donors (Table I).

Relationship Between HHV-6 Reactivation and Disease Activity in MS

As shown in Table I, 7/11 (63.6%) patients with relapsing/remitting MS and 9/15 (60.0%) patients with secondary progressive MS had HHV-6 sequences in PBMC DNA. Plasma samples from these 16 patients (20 samples in total) were obtained during periods of disease activity (exacerbation) and quiescence (remissions/relative remissions). HHV-6 DNA was detected in four of seven plasma samples (4 of 6 patients) obtained during an exacerbation but was not found in four plasma samples (4 patients) obtained during remission of relapsing/remitting MS (Table II). Similar results were obtained from the examination of plasma samples of the nine patients with secondary progressive MS. The viremia was found only during disease exacerbation, including the presence of Gd-enhancing lesions on MRI (4 of 7 patients), and not during relative remission with an absence of Gd-enhancing lesions on MRI (0 of 2 patients) (Table II).

Thus, HHV-6 viremia was detected in 8/16 (50.0%) patients with MS and with evidence of latent HHV-6 infection and was only detectable during exacerbation of both relapsing/remitting and secondary progressive MS (8 of 13; 61.5%).

PBMC RNA and plasma samples of the five patients with MS, two relapsing/remitting (Patients 4 and 7) and three secondary progressive (Patients 5–7), negative for HHV-6 viremia during disease activity were additionally studied by RT-PCR and IFA (Table III). Transcription of viral mRNA, which indicates early HHV-6 replication, was found in 2/3 PBMC RNA samples from

TABLE II. Examined of Relapsing/Remitting MS and Secondary Progressive MS Patients With HHV-6 Infection*

Patients	Phase of MS	HHV-6 infection (nPCR)		MRI[a]	IL-12 pg/ml (ELISA)
		PBMC	Plasma		
Relapsing/remitting MS					
1	Exacerbation	+	+	+	27.3
2	Exacerbation	+	+	+	34.0
3	Remission	+	−	−	<5.0
	Exacerbation	+	+	+	23.1
4	Exacerbation	+	−	+	14.0
	Remission	+	−	−	<5.0
5	Remission	+	−	−	<5.0
6	Remission	+	−	−	<5.0
	Exacerbation	+	+	+	14.1
7	Exacerbation	+	−	+	6.0
	Exacerbation	+	−	+	8.0
Secondary progressive MS					
1	Exacerbation	+	+	+	21.0
2	Exacerbation	+	+	+	18.0
3	Relative remission	+	−	−	11.4
4	Exacerbation	+	+	+	23.1
5	Exacerbation	+	−	+	12.0
6	Exacerbation	+	−	+	9.4
7	Exacerbation	+	−	+	12.2
8	Exacerbation	+	+	+	16.3
9	Relative remission	+	−	−	8.3

*MS, multiple sclerosis; PBMC, peripheral blood mononuclear cells; MRI, magnetic resonance imaging; (+), positive results; (−), negative results.
[a]MRI results: (+), presence of Gd-enhancing lesions; (−), absence of Gd-enhancing lesions.

TABLE III. Presence of HHV-6 mRNA and Virus-Specific Antibodies in MS Patients With Disease Exacerbation and Nondetectable Viremia*

		HHV-6 infection[a]				
		nPCR			IFA	
Patients	Phase of disease	PBMC	Plasma	RT-PCR PBMC	IgM	IgG (titer)
Relapsing/remitting MS						
4	Exacerbation	+	−	+	+	ND
7	Exacerbation	+	−	+	+	ND
	Exacerbation	+	−	−	−	+ (1:60)
Secondary progressive MS						
5	Exacerbation	+	−	−	−	+ (1:40)
6	Exacerbation	+	−	−	−	+ (1:20)
7	Exacerbation	+	−	−	−	+ (1:60)

*MS, multiple sclerosis; PBMC, peripheral blood mononuclear cells; nPCR, nested polymerase chain reaction; RT-PCR, reverse transcriptase polymerase chain reaction; IFA, indirect immunofluorescence assay.
[a](+), positive results; (−), negative results.

patients with relapsing/remitting MS. Active viral infection in these patients was confirmed by the detection of specific anti-HHV-6 IgM antibodies in their plasma (Table III). Neither transcription of viral mRNA in PBMC RNA, nor the presence of IgM specific antibodies or elevated titers of IgG antibodies to HHV-6 in the plasma (1:20–1:60) of three patients with secondary progressive MS were found, confirming that in these patients HHV-6 infection remained latent.

Viral mRNA transcription in PBMC RNA and specific IgM antibodies in plasma were not detected in any of the six patients with other neurological disorders and the titers of IgG antibodies to HHV-6 did not exceed the range 1:20–1:80.

The plasma samples from the patients with exacerbation of MS, in whom HHV-6 viremia was demonstrated, were not examined for the antibodies to HHV-6. Thus, reactivation of HHV-6 was detected in 10/16 (62.5%) patients with MS and latent HHV-6 infection and was found only in during periods of disease activity in both relapsing/remitting and secondary progressive MS (10/13; 76.9%). The risk of an exacerbation of MS calculated according to the type of HHV-6 infection (active or latent) was 2.5 times higher for the patients with HHV-6 reactivation ($P = 0.005$).

Relationship Between Serum Level of IL-12, Disease Activity In MS and HHV-6 Reactivation

An IL-12 concentration of greater than 5 pg/ml was found in 16/20 (80.0%) serum samples from patients with MS (Table II). IL-12 was not detected, in the six serum samples examined, in patients with other neurological disorders. In relapsing/remitting MS the mean serum IL-12 concentration was 18.07 ± 2.65 pg/ml in the active phase of the disease (7 serum samples from 6 patients), but IL-12 was not detectable in the remission phase (4 samples from 4 patients) (Table II). In all nine patients with secondary progressive MS IL-12 was detectable, mean serum IL-12 concentration 14.63 ± 1.74 pg/ml (Table II). The IL-12 level was 1.6-fold higher (16.0 ± 1.85 pg/ml) in the seven patients with "active disease" than in the two patients with "quiescent disease" (9.85 ± 3.4 pg/ml), although the difference was not statistically significant.

Among the patients with active MS the mean serum IL-12 concentration was significantly higher ($P < 0.05$) in the 10 patients with active HHV-6 infection compared to the three patients with latent viral infection (19.89 ± 3.36 and 9.90 ± 1.45 pg/ml, respectively). In patients with active HHV-6 infection and active MS no significant difference was found in serum IL-12 concentrations according to whether they had relapsing/remitting MS or secondary progressive MS (20.08 ± 3.08 and 19.60 ± 1.02 pg/ml, respectively).

DISCUSSION

Although some studies have failed to show an association between HHV-6 and MS [Campadelli-Fiume et al., 1999; Hay and Tenser, 2000; Taus et al., 2000], these findings support a correlation between HHV-6 and MS as reported earlier both by ourselves [Tomsone et al., 2001] and others [Soldan et al., 1997; Ablashi et al., 2000; Akhyani et al., 2000]. The prevalence of HHV-6 infection was significantly higher in patients with MS (61.5%) compared to patients with other neurological disorders (28.6%, $P = 0.039$) and the control blood donors (28.7%, $P = 0.0027$); however, this association did not provide evidence that HHV-6 was involved in the pathogenesis of MS.

The aim of this study was to establish whether a relationship existed between HHV-6 reactivation and disease activity in MS. HHV-6 reactivation, measured by plasma viremia, transcription of viral mRNA in PBMCs and serology was correlated with patterns of disease activity and serum IL-12 concentrations in 16 patients with MS. Disease activity in MS was classified as active (clinical exacerbation and active inflammatory lesions on Gd-enhanced MRI) or quies-

cent (clinical remission in relapsing/remitting MS or relative stability in secondary progressive MS and the absence of active inflammatory lesions on Gd-enhanced MRI).

Our results (Table II) confirm the relationship between the active lesions on MRI scans and patterns of disease activity in MS shown previously [Filippi, 1998; Weiner et al., 2000]. The presence of HHV-6 DNA in blood plasma, which is a marker of active viral infection [Secchiero et al., 1995], was detected only in patients with MS, all of whom had latent infection detected previously, and not in patients with other neurological disorders nor in blood donors (Tables I, II). This viremia was observed only during periods of new disease activity, both in relapsing/remitting and secondary progressive MS (8/13 patients; 61.5%) (Table II). These results are in accordance with those of Soldan et al. [1997], Akhyani et al. [2000], and Ablashi et al. [2000] who reported a higher PCR positivity for HHV-6 in plasma of patients with MS. The absence of HHV-6 viremia in some patients with active MS may be associated with an early stage of viral replication [Secchiero et al., 1995]. RT-PCR assay is more sensitive for the detection of an early stage of HHV-6 activation than DNA PCR in plasma and can be used reliably to differentiate between the latent and active HHV-6 infection [Norton et al., 1999]. Using RT-PCR, we found that 2/5 PBMC RNA samples from patients with active relapsing/ remitting MS but without detectable viremia, were positive for HHV-6 transcripts (Table III). Active viral infection was confirmed by the presence of HHV-6-specific IgM antibodies in the plasma of these patients (Table III). Thus, clinical and MRI activity in MS was observed simultaneously with the HHV-6 reactivation in 76.9% cases (10/13 patients).

The evidence that IL-12 is involved in the pathogenesis of MS is accumulating. We have demonstrated elevated IL-12 production in 80.0% of patients with MS but not in patients with other neurological disease. Similar findings have been reported elsewhere [Nicoletti et al., 1996; Drulovic et al., 1998; Matusevicius et al., 1998]. Furthermore, a clear correlation between the serum IL-12 concentration and clinical/MRI activity in relapsing/remitting MS patients was demonstrated. IL-12 in the sera of these patients was detectable in the active phase of the disease only (Table II). The patients with secondary progressive MS had detectable serum IL-12 during periods of relative stability as well as during exacerbation (Table II); however, the mean concentration of this cytokine was 1.6-fold higher during exacerbation ($P > 0.05$). Other researchers have also found an association between an increase of IL-12 concentration in sera [Nicoletti et al., 1996; Drulovic et al., 1998; Matusevicius et al., 1998], an increase in IL-12 production by monocytes in untreated MS patients [Comabella et al., 1998] an increase of IL-12 p40 mRNA [van Boxel-Desaire et al., 1999] and disease activity in MS. In contrast to previous studies [Balashov et al., 1997; Heesen et al., 1999] we did not observe a significant difference between serum levels of IL-12 in relapsing/remitting MS and secondary progressive MS patients when both groups were in clinical exacerbation with active lesions on MRI.

A clear correlation between HHV-6 reactivation and increased serum IL-12 concentrations during disease activity in MS has been demonstrated for the first time. The concentrations of this cytokine in sera were significantly higher ($P < 0.05$) in MS patients with active HHV-6 infection compared to the patients with latent HHV-6 infection, although both groups (with and without active viral infection) were in the active phase of the disease (Table II). These data suggest that HHV-6 reactivation has a stimulatory effect in vivo on IL-12 synthesis. The capacity of HHV-6 to induce immunodisregulation through the alteration of cytokine production has been shown in vitro [Arena et al., 1999].

The clear correlation between HHV-6 reactivation, increased serum IL-12 concentration and disease activity in relapsing/remitting MS and secondary progressive MS suggests that HHV-6 reactivation is involved in the exacerbation of this disease. The risk of MS exacerbation is significantly higher (odds ratio 2.5; $P < 0.005$) in patients with HHV-6 reactivation in comparison to patients with latent HHV-6 infection.

Several recent studies have shown that heterogeneous pathogenic mechanisms may lead to inflammatory demyelinating plaques in MS patients. Because HHV-6 infects cells of the immune system, it may modulate their functions. It can be postulated that the activation of the virus induces demyelination by a variety of immune mechanisms via selective regulation of cytokine synthesis. Taking into account the ubiquitous nature of HHV-6, its interaction with the newly discovered human endogenous MS-associated retroviruses (HERV-H and HERV-W) [Perron et al., 1997; Christensen et al., 2000] cannot be excluded.

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