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Short Communication

A pilot study on the antibodies to HHV-6 variants and HHV-7 in CSF of MS patients

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In the possible role for human herpesviruses (HHV) in the pathogenesis of multiple sclerosis (MS) neither clear distinction between the two variants of HHV-6, nor the involvement of HHV-7 have been described. Therefore, we quantitated HHV-6 variant specific and HHV-7 reacting antibodies in the CSF of 13 patients with MS or other neurological disorders by ELISA. Predominance in the positivity of IgG (67%) and IgM (44%) to HHV-6B over that of IgG (44%) with no detectable IgM to HHV-6A, and no antibodies to HHV-7 were found in the CSF of MS patients. None of these antibodies were found in the CSF of controls. This suggests that, intrathecal chronic active or primary HHV-6B infection might contribute to MS progression, while the local effects of HHV-6A and HHV-7 seem to be less important.

Keywords: MS; CSF; antibodies; ELISA; HHV-6B; HHV-7

Multiple sclerosis is one of the most common neurological disorders in Europe and North America (Bencsik *et al*, 1998; Perron *et al*, 1997). There is serological and molecular evidence that, human herpesvirus type 6 (HHV-6) might have a role in the pathogenesis of MS (Sola *et al*, 1993), but studies on the involvement of HHV-7 in MS, which is closely related to HHV-6 have not been published. Isolates of HHV-6 are grouped as variants A and B, and the contradictory results using different serological and molecular assays (Fillet *et al*, 1998; Soldan *et al*, 1997) raises the possibility that HHV-6A and HHV-6B might exert different effects in disease progression. Recent reports indicate that, HHV-6A and HHV-6B have distinct biological properties and pathogenic potentials. In an individual, no cross-immunity exists between HHV-6A and HHV-6B despite their genomic similarity (Hall *et al*, 1998). In spite of the integrity of the blood–brain barrier (BBB) HHV-7 (Portolani *et al*, 1998), both HHV-6A (Hall *et al*, 1998) and HHV-6B (Challoner *et al*, 1995) may reach the central nervous system (CNS). In children and adults with dual infections, only HHV-6A persists in CSF, which suggests that HHV-6A had greater neurotropism, while HHV-6B tends to be more prominent in other tissues (Hall *et al*, 1998; Luppi *et al*, 1994). In contrast, the presence of HHV-6B sequences is found to be common in the brains from MS patients and controls, and the high degree expression of HHV-6B in the plaque-associated oligodendrocytes (which are destroyed throughout disease course) suggests that rather local than general effects of viruses contribute to MS pathogenesis (Challoner *et al*, 1995). Detection by polymerase chain reaction (PCR) without HHV-6 variant specificity in peripheral blood mononuclear cells did not show any significant difference in frequency and quantity between MS patients and controls. It is suggested that brain cells are the reservoir for the virus (Mayne *et al*, 1998). Both HHV-6 (Patnaik *et al*, 1995) and HHV-7 (Torigoe *et al*, 1996) induce intrathecal synthesis of IgM and IgG. Until the breakdown of BBB in the later stage of disease, variant specific antibodies primarily in the cerebrospinal fluid (CSF) (Patnaik *et al*, 1995) and secondarily in the serum (Ablashi *et al*, 1998; Nielsen *et al*, 1997; Sola *et al*, 1993; Soldan *et al*, 1997; Wilborn *et al*, 1994) might correlate with disease progression.

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For our present studies patients with MS and other neurological disorders (OND) were diagnosed by standard clinical and laboratory criteria (Bencsik *et al*, 1998; Ungurean *et al*, 1996). Cerebrospinal fluid (CSF) and serum of patients obtained with prior informed consent were subjected to low speed centrifugation to establish leukocyte and erythrocyte numbers. Protein content, albumin and immunoglobulin (Ig) fractions were quantitated by laser nephelometry (Dosatec GmbH, Munich, Germany) and isoelectric focusing in an agarose gel as described earlier (Bencsik *et al*, 1998; Seres *et al*, 1998; Ungurean *et al*, 1996). The mean of CSF/serum albumin index was 4.95×10^{-3}. This was within the normal range showing no BBB damage. Two exceptions were Patients 7 and 11, who also had erythrocytes in the CSF. We used a sensitive ELISA to quantitate variant specific IgG and IgM, as described (Maródi *et al*, 1998). Briefly, JJHAN cells infected with HHV-6A GS, MOLT-3 cells infected with HHV-6B Z29, Sup-T1 cells infected with HHV-7 RK strain served as antigens. When OHV-1 monoclonal antibody (MAb; Advanced Biotechnologies Inc., Columbia, MD, USA) to both HHV-6 variants and RK-4 MAb to HHV-7 in indirect immunofluorescent assays detected equal ratio (38–40%) of cells containing viral antigens (data not shown), these cells were fixed to 96 well polystyrene plates. HHV-6 variant specificity was verified by competitive binding of anti-HHV-6B 101k MAb (PE Pellett, Atlanta, GA, USA). Competing IgG was removed from aliquots of each serum and CSF by mixing them with Protein-A Sepharose 4B (Sigma, St. Louis, MO, USA) in 10 : 1 ratio for 1 h with subsequent low speed centrifugation. Twofold dilutions of sera and CSF (1 : 100–1 : 6400) were incubated with the antigens in 96 well polystyrene plates in quadruplicate. After vigorous washings, peroxidase conjugated anti-human IgG and IgM (Sigma), respectively, were adsorbed to human polypeptides in parallel wells, finally orthophenyldiamine (OPD) substrate was introduced in each well to detect enzyme in the immune complexes. The optical density, read at 492 nm, >150% higher than that of the standard deviation of established seronegative subjects were regarded as positive. Sera of known seropositive individuals (kindly provided by L Ceccherini-Nelli, Pisa, D DiLuca, Ferrara, Italy) served as controls. Serum and CSF dilutions at 1 : 100 were tested in quadruplicate for non-specific binding to uninfected cells, but no reactions were detected (data not shown).

In MS patients, oligoclonal bands, protein content, nephelometry and presence of variant specific antibodies showed a significant correlation (Table 1). Predominance in the positivity of IgG (6/9=67%) and IgM (4/9=44%) to HHV-6B over IgG (3/9=33%) with no detectable IgM to HHV-6A was found. This raises the possibility that a previous intrathecal HHV-6A infection silent at time of the test, and chronic active or primary HHV-6B infection (especially in Cases 2, 5, 6 and 7) might contribute to MS. On the contrary, lack of HHV-7 specific antibodies in CSF argues against its direct role in MS. As seven of nine patients had antibodies to HHV-7 in their sera (data not shown), this also demonstrated integrity of BBB. Presence in CSF, but absence in serum of IgM antibodies to HHV-6B found in Patient 7 with damaged BBB suggests its intrathecal synthesis. No CSF antibodies to HHV-6 variants or HHV-7 were found in the youngest MS patients (Patients 1 and 5), which might suggest involvement of another agent(s) in triggering disease. In patients with OND, CSF contained no detectable antibodies

Table 1 Antibodies to HHV-6 and HHV-7 in CSF of patients with MS and other neurological disorders (ELISA)

Number of patients	*Age (years)*	*Sex (M/F)*	*Diagnosis*	*Leukocytes (10^6/L)*	*Protein (g/L)*	*Nephelometry*	*Oligoclonal bands*	*HHV-6A*		*HHV-6B*		*HHV-7*	
								IgG	*IgM*	*IgG*	*IgM*	*IgG*	*IgM*
								antibody titres+					
Multiple sclerosis													
1	25	M	RR	1	0.26	–	+	Ø	Ø	Ø	Ø	Ø	Ø
2	29	M	RR	45	0.38	IgG, IgM	+	Ø	Ø	800	400	Ø	Ø
3	34	M	RR	0	0.22	IgA, IgG, IgM	+	400	Ø	100	Ø	Ø	Ø
4	53	M	RR	1	0.54	IgG (traces)	+	Ø	Ø	100	Ø	Ø	Ø
5	34	F	RR	0	0.26	IgG	+	Ø	Ø	200	200	Ø	Ø
6	36	F	FA	0	0.41	IgA, IgM	–	200	Ø	200	100	Ø	Ø
7	41	F	RR	5*	0.20	IgG	+	400	Ø	800	200	Ø	Ø
Other neurological disorders													
8	42	M	IDR	0	0.40	IgM	–	Ø	Ø	Ø	Ø	Ø	Ø
9	43	M	CHA	0	0.39	ND	–	Ø	Ø	Ø	Ø	Ø	Ø
10	50	M	CHA	1	0.30	–	–	Ø	Ø	Ø	Ø	Ø	Ø
11	1	F	AHE	100**	0.86	IgA, IgM, IgG (traces)	–	Ø	Ø	Ø	Ø	Ø	Ø
12	25	F	PNP	0	0.17	IgG, IgM	+	Ø	Ø	Ø	Ø	Ø	Ø
13	22	F	PNP	21	0.47	IgG	+	400	Ø	Ø	Ø	Ø	Ø

+Reciprocal values of serum dilutions; RR=relapsing-remitting; FA=first attack; IDR=intervertebral disk rupture; CHA=chronic headache; AHE=acute HSV-1 encephalitis; PNP=polyneuropathy; ND=not done; *64 and **1024 erythocytes (10^6/L), Ø=<100

to HHV-6 or HHV-7. The only exception was Patient 13 without BBB damage, who also had low level serum IgG to HHV-6A. The mean titers of the two patient groups were not significantly different. Patient 11 was seronegative for HHV-6 variants and HHV-7, and herpes simplex virus type 1 (HSV-1) was identified as a causative agent of encephalitis.

In the serum of patients with MS and OND, as well as 12 healthy subjects, IgM antibody titers to HHV-6A were not different, and a slight, statistically non-significant increase in IgG titers of both patients groups as compared to the normal individuals was observed. The mean anti-HHV-6B IgG and IgM levels were significantly higher in the MS group than in OND and healthy subjects. This difference was less than that found in the CSF of MS patients compared to OND patients. No IgM to HHV-7 was found in the serum of any persons studied, and even the mean IgG titer of healthy controls was slightly higher than that of the two patients groups. Details will be described elsewhere.

Presence of CSF antibodies in different titers suggests different roles of HHV-6 variants, while the absence of CSF antibodies to HHV-7 seems to exclude its intrathecal contribution to the pathogenesis of MS. In few cases, detection of low level IgG without IgM to HHV-6A indicates past infection without recent expression of viral genes. On the contrary, in nearly two thirds of patients the simultaneous presence of IgG and IgM to HHV-6B suggests ongoing or preceding intrathecal replication of HHV-6B. So far, CSF antibodies have been studied occasionally, but the comparison of serum antibodies with variant specific PCR might support our observations. Recently, in the sera of patients with exacerbation of MS, increased IgM response to the common early polypeptides p38/41 of HHV-6 variants in the absence of increased IgG levels has been found (Soldan *et al*, 1997), which indicates persistent active infection by any of the variants. Primers unable to differentiate between two variants detected HHV-6 in the peripheral blood mononuclear cells (PBMC) of 30% of the same patients (Soldan *et al*, 1997). In another study, IgG and IgM to p38/41 were found in the serum of 68 and 56% of MS patients by ELISA, respectively. Although this also suggests active infection without variant definition, PCR identified HHV-6B DNA sequences in the PBMC of the same patients (Ablashi *et al*, 1998). If HHV-6A GS was used in an ELISA, no difference between IgG titers in MS patients and controls were found (Nielsen *et al*, 1997), that also excludes recent infection by this variant. Using a HHV-6A-like strain, ELISA revealed a significantly higher total serum level of IgG+IgM than in controls or OND patients, but neither IgG+IgM nor HHV-6A DNA were found in any of their CSF samples (Wilborn *et al*, 1994). Significantly higher anti-HHV-6A (GS) IgG titers in MS patients in comparison with the blood donors were found, but only 7% of MS patients without BBB damage had high IgG level in their CSF. In one of 31 MS patients high, in one of 24 controls low copy number of HHV-6A DNA in their PBMC were found, both were negative for serum IgG. These patients might have been positive for IgM, which was not tested, or their IgG level was under the limit of detection as the less sensitive IFA was used (Sola *et al*, 1993). Similarly, different pathogenetic mechanisms can be assigned to HHV-6 variants in HIV-1 infected patients, where DNA of HHV-6A is detected in PBMC, while that of HHV-6B is shown in different organs (Emery *et al*, 1999).

More recently, an MS-associated retrovirus (MSRV) has been isolated, characterized and suggested as another etiological agent (Perron *et al*, 1997). HHV-6, EBV or HSV-1 might behave as cofactors, and exert their effects in MS through transactivating MSRV (Perron *et al*, 1993). This mechanism is similar to the HIV transactivation by HHV-6A in AIDS progression (Ongrádi *et al*, 1990) or enhancing lymphomagenesis by HHV-6B (DiLuca *et al*, 1994) via altering cytokine profile. Up to date, no such role has been found for HHV-7 in these clinical phenomena. HHV-6B might, HHV-6A and HHV-7 might not induce local abnormalities in the intrathecal synthesis of cytokines and therefore affect MSRV and autoimmune demyelination. As the clinical outcome seems to be variant specific, in further reports, therefore, a more clear distinction of HHV-6 variants studied must be declared.

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