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Extended observations on the association of HHV-6 and multiple sclerosis

Rossana Berti[1], Samantha S Soldan[1,2,3], Nahid Akhyani[1], Henry F McFarland[1] and Steven Jacobson*[,1]

[1]Viral Immunology Section, Neuroimmunology Branch, National Institutes of Health, Bethesda, Maryland, MD 20892, USA; [2]Institute of Biomedical Sciences, George Washington University, Washington, DC 20052, USA and [3]Department of Genetics, George Washington University, Washington, DC 20052, USA

Throughout the years, a long list of viruses has been associated with multiple sclerosis (MS), however no virus to date has been definitively identified as the etiologic agent of this disease. Recently, human herpesvirus 6 (HHV-6), a newly described herpesvirus, has been suggested to play a role in MS based on: immunohistochemical demonstration of HHV-6 in MS plaques, increased antibodies response to HHV-6 in sera and CSF of MS patients, and the demonstration of HHV-6 DNA in the serum of MS patients but not in normal individuals. To extend these observations we have focused our research in multiple directions. We have increased the number of MS patients tested for HHV-6 serum DNA providing confirmation of our previous study. Additionally we have investigated a possible correlation between HHV-6 viremia and clinical activity. Finally to provide insight into the pathogenesis of this disease, we have begun to characterize the cellular immune response of MS patients to HHV-6. Collectively these studies will help to define the role that HHV-6 may play in the pathogenesis of MS. *Journal of NeuroVirology* (2000) **6,** S85–S87.

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Viruses have long been suggested to be involved in the etiology of multiple sclerosis (MS) (Berti and Jacobson, 1999; Johnson, 1994). However, while the search for a single pathogen as a causal agent has failed to identify a unique virus associated with this disorder, it has been suggested that a common or ubiquitous virus may act as a trigger for this disease depending on genetic influences and host-virus specific cellular immune responses.

Recently, human herpesvirus 6 (HHV-6), a newly described β-herpesvirus (Salahuddin *et al*, 1986), has been suggested to play a role in MS (Challoner *et al*, 1995; Sola *et al*, 1993; Soldan *et al*, 1997). HHV-6 is the etiologic agent of *Exanthem Subitum* (*Roseola Infantum*) (Yamanishi *et al*, 1988) and up to 95% of the adult population has been exposed to it (Braun *et al*, 1997). Its genome is about 160 Kb long and it is likely to encode 80–100 polypeptides (Lusso and Gallo, 1995). On the basis of genetic restriction patterns and cellular tropism HHV-6 isolates are classified into two variants: A and B. The sequence homology between them varies from 75–96% depending on the loci considered (Lusso *et al*, 1995). In the past few years, HHV-6 has been shown to be highly neurotropic and its role in the Nervous System has been demonstrated in many works from different groups (Caserta *et al*, 1994; Mackanzie *et al*, 1995; Sanders *et al*, 1996; Wilborn *et al*, 1994).

HHV-6 is considered to be a possible candidate in the pathogenesis of MS for different reasons: (1) Primary infection with HHV-6 usually occurs during the first 2 years of life and would be consistent with epidemiological evidence in MS suggesting childhood exposure to an etiologic agent; (2) Herpes viruses, in general, are highly neurotropic; (3) One of the fundamental properties of herpesviruses is their tendency to reactivate. The same factors that often lead to herpesvirus reactivation, such as stress and infection with another agent have also been associated with MS exacerbations and (4) while HHV-6 is predominantly a T-lymphotropic virus, it has also been found to infect other cells of both lymphoid and non-lymphoid origin. HHV-6 is a pleiotropic virus, which infects cells of both the immune and nervous systems. As such, the virus could cause pathology in both. We

*Correspondence: S Jacobson, NIH, NINDS, NIB, Building 10, Room 5B16, 10 Center Drive, Bethesda, Maryland, MD 20892, USA

Table 1 Prevalence of IgM antibodies to HHV-6 in the serum of MS patients from three different studies: UCLA (WW Tourttellotte, unpublished observations); NIH (ref 13); Rockefeller University (Friedman, submitted)

Samples	*Number tested*	*Positive (%)*	*Group*
Normals	11	18	UCLA
Multiple sclerosis	14	71	UCLA
Normals	11	18	NIH
Multiple sclerosis	22	73	NIH
Normals	19	16	Rockefeller
Multiple sclerosis	25	80	Rockefeller

(Soldan *et al*, 1997) have recently demonstrated increased IgM response to the p41/38 antigen of HHV-6 in relapsing remitting MS (RRMS) patients (73%) compared to normal donors (ND) (18%). These data are supported by data from two other groups: a group at UCLA (WW Tourtellotte, unpublished observations) reported increased IgM response in 71% of MS patients compared to 18% ND; another group at Rockefeller University (Friedman *et al*, 1999), using a different assay, found a higher response in MS patients compared to ND (80% and 16% respectively) (Table 1). The lack of an IgM response to two closely related herpesviruses (CMV and EBV) indicates that the response to the early HHV-6 antigen p41/38 is specific and not the result of generalized increased IgM production (Soldan *et al*, 1997).

To support these observations we have looked for the detection of HHV-6 DNA in sera of MS patients. The presence of HHV-6 DNA in the serum is a marker of active infection and has previously been reported in children with roseola and in some immunocompromised patients, but not in normal donors (Secchiero *et al*, 1995). Using a highly sensitive nested PCR assay (Secchiero *et al*, 1995) with primers to the major capsid protein (MCP) region of HHV-6 we looked for the viral DNA in the sera of MS patients. In our paper (Soldan *et al*, 1997) we reported the presence of HHV-6 DNA in 30% (15/50) of MS patients tested. The same DNA was not found in the sera of control patients. We have now extended these observations to a total of 77 MS individuals and we have found the presence of HHV-6 DNA in the same percentage of MS patients (Jacobson *et al*, 1998).

After the demonstration of HHV-6 reactivation in MS patients we decided to study a possible correlation between HHV-6 viremia and clinical findings. We started a prospective study: patients with MS enrolled in the clinical protocols at the neuroimmunology branch of the NINDS at NIH were followed for a study period of 5 months. Using a nested polymerase chain reaction, they were tested for the presence of HHV-6 reactivation and afterwards they were correlated with their disease activity.

During those 5 months more than 50% of the patients experiencing an exacerbation had detectable level of HHV-6 DNA in their sera. The same DNA was also detected in some of the patients without clinical change at the time of the viremia. For one patient with secondary progressive MS and frequent period of clinical worsening, serum samples were retrospectively analyzed for HHV-6 DNA. Over a period of 18 months we were able to detect HHV-6 DNA during more than 60% of his exacerbations. The same DNA was detected only once during clinical absence of exacerbations (RB, unpublished observations).

As mentioned previously, there are two variants of HHV-6: A and B. Variant B is divided into two subgroups, B1 and B2. The gel represented in Figure 1 is obtained amplifying the DNA from different HHV-6 subtypes for the large tegument protein region and digesting the nested PCR product with three enzymes (*Hind*III, *Bgl*II and *Taq*I). The three different restriction patterns allow us to distinguish between variant A, B1 and B2. To establish which specific variant is present in our patients and if there is a difference between healthy and MS individuals we have started to isolate HHV-6 DNA from body fluids including serum, urine, saliva. Our prelminary results indicate that a significant percentage of HHV-6A can be detected in acellular material such as serum and urine from MS patients but not healthy controls. In contrast, the frequency

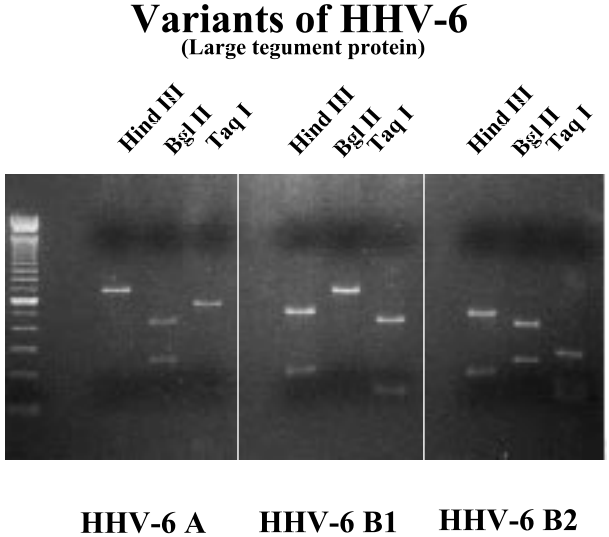

Figure 1 Enzymatic digestion of nested PCR product obtained amplifying three different HHV-6 subtypes for the large tegument protein region: lane 1) 100 bp ladder; lanes 2–4) enzymatic digestion of HHV-6 A respectively with *Hind*III, *Bgl*II and *Taq*I; lanes 5–7) enzymatic digestion of HHV-6 B1 respectively with *Hind*III, *Bgl*II and *Taq*I; lanes 8–10) enzymatic digestion of HHV-6 B2 respectively with *Hind*III, *Bgl*II and *Taq*I.

of HHV-6 detection in PBL and saliva was similar in patients and controls in which the virus was characterized as predominantly HHV-6B.

To provide insight into the pathogenesis of this disease, we have started to characterize the cellular immune response of MS patients to HHV-6. PBL from both ND and MS patients were stimulated with lysates of uninfected T-cell lines and T-cell lines infected with the two variant of HHV-6 (A and B) and HHV-7. Lymphoproliferative response to HHV-6 A, B and HHV-7 could be demonstrated both in ND and MS. The majority of ND responded to HHV-6B, however a higher percentage of patients with MS compared to ND gave a response to HHV-6 A (RB, unpublished observation).

Infectious agents have been implicated as the cause of MS for over a century. However no definitive association has been made and caution is warranted when correlating viral agents with this disorder. Although our data suggest a possible role of HHV-6 in the pathogenesis of MS, further studies are still necessary.

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