plaques with the same monoclonal antibody (Chemicon International) to HHV-6B 101K virion protein. In our hands this antibody detected virion protein in tissue sections of HHV-6 infected salivary gland tissue[7] but was negative for plaques from the brains of 23 MS patients (ten frozen and 13 paraffin embedded samples). We then isolated RNA from the same plaques in the ten frozen MS specimens and from the frozen brains of 35 individuals (frozen brains kindly supplied by P. Ince, MRC Neurochemical Pathology Unit and R Ravid, Netherlands Brain Bank) who had died from non-MS diseases. RT-PCR was performed, again blinded, with primers derived from the *U11* and *U31* (Genbank Acc.No. X83413) of HHV-6A. We found positive signals in HHV-6 viral cultures and no signal in the negative control in which the reverse transcription step was omitted. Although RT-PCR with these primers gave a positive result in 24 out of the 45 brains, there was no significant difference between the number or the intensity of the signals in MS and non-MS brains.

Our data do not support an association between HHV-6 and MS. Contradictions to earlier work may be due to patient heterogeneity, the fact that we studied different genes or because we studied post-mortem brain samples in contrast to Soldan *et al.* who studied sera.

ANTHONY R.M. COATES[1] & JEANNE BELL[2]
[1]*Division of Molecular Microbiology*
St George's Hospital Medical School
Cranmer Terrace, London SW17 ORE, UK
[2]*Neuropathology Laboratory*
Department of Pathology
The University of Edinburgh
Western General Hospital
Edinburgh EH4 2XU, UK

Soldan et al. reply—We agree with Fillet *et al.* that serological studies in MS are difficult to interpret due to defects in immunoregulation which often result in increased antibody titers in MS patients. However we demonstrated that the IgG and IgM antibody responses of MS patients to two other herpesviruses, CMV and EBV, were not significantly different from controls, suggesting that the elevated anti-HHV-6 p41/38 IgM antibody response in patients with relapsing remitting MS are not the result of generalized immunodisregulation.

Our antibody studies were supported by molecular analyses. Previously, serum HHV-6 DNA has only been described in individuals with primary HHV-6 infection (*exanthem subitum*) and in immunocompromised individuals[8]. Therefore, the presence of viral DNA in serum is indicative of an active HHV-6 infection. Using a sensitive nested PCR technique, we described the presence of HHV-6 serum DNA in 30% (15 of 50) of MS patients, suggesting viral reactivation in a subset of patients. Fillet *et al.* attempted to detect viral DNA in CSF serum of MS patients using a simple primary PCR procedure followed by a non-radioactive hybridization microplate assay. Both their work and ours shows that HHV-6 DNA can not be detected in the sera of MS patients by primary PCR alone. Unfortunately, the methods for both DNA extraction[9] and amplification used by Fillet *et al.* were quite different from ours. Indeed the amplification protocol used by Fillet *et al.* was developed for detecting HHV-6 DNA in peripheral blood mononuclear cells (PBMCs) and saliva[9] and not from serum. The primers we used were also different. These differences in PCR design, extraction procedures, primers and patient populations make direct comparisons between our two studies difficult.

Table 2 PCR- detection* of HHV6 DNA in serum

Patients	Positive Serum	Negative Serum
Multiple Sclerosis	21#	56
Normal Donors	0	19
Other Neurologic Diseases	0	19
Other Inflammatory Diseases	0	15

*DNA extraction and nested PCR were as previously described[1]. This assay can detect as little as 0.45 fg of DNA. # MS patients compared to all other samples, $p < 0.0001$ (Chi-square).

We have continued to examine additional MS patient sera for HHV-6 DNA. To date, 77 MS patients have been examined (Table 2) and we continue to find HHV-6 DNA in a comparable subset (27%) of these patients.

An additional concern raised by Fillet *et al.* is that immunosuppression as a result of corticosteroid therapy may be influencing the reactivation of HHV-6. However, the MS patients used in our study did not receive corticosteroids for at least two months prior to testing of their serum for HHV-6 DNA. This is in contrast to the therapeutic strategy used in patients with other inflammatory diseases (OID). The majority of patients in the OID cohort were on long term high dose steroid treatment and no serum HHV-6 DNA could be detected in these patients (see ref. 1 and Table 2). We have also obtained blood from patients previously found to be negative for serum HHV-6 DNA and subsequently treated with methylprednisone. HHV-6 DNA remained undetected in these patients, indicating that corticosteroid therapy is not associated with HHV-6 reactivation.

Coates and Bell describe *in situ* studies from MS autopsy material in which they were unable to detect HHV-6 protein or RNA, by immunohistochemical and RT-PCR assays. While their work does attempt to reproduce that of Challoner *et al.*[2] it has no direct bearing on our recent paper[1]. However, the points they raise are important and we have also begun to evaluate the role of HHV-6 in MS neuropathology. Recent data generated by our group indicates the presence of HHV-6 protein by immunohistochemistry in a small number of MS autopsy brain specimens (paraffin embedded) compared to a panel of other disease controls (including brains with blood brain barrier breakdown and inflammation) . HHV-6 protein was detected in active MS lesions. Furthermore, by using a double staining technique, we are able to identify neuroglial cells expressing HHV-6 protein within active MS lesions, in one extensively studied case (manuscript submitted).

STEVEN JACOBSON, SAMANTHA S. SOLDAN & ROSSANA BERTI
Viral Immunology Section
National Institutes of Health
Building 10, Room 5B-16
Bethesda, MD 20892, USA
stevej@helix.nih.gov

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