

Role of Viral Infection in the Aetiology of Multiple Sclerosis

Status of Current Knowledge and Therapeutic Implications

Rossana Berti and Steven Jacobson

Viral Immunology Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, USA

Abstract

Multiple sclerosis (MS) is a chronic disease of the CNS that typically begins in late adolescence or early adulthood. It is highly variable in its expression and severity. The cause of MS is unknown, but both genetic and environmental factors have been implicated in its pathogenesis. It is known that viruses can induce chronic neurological disease, but the pathogenetic process is unclear. A viral cause for MS has been postulated, but to date no single virus has been confirmed to be associated with the disease. Although most viral candidates are no longer considered as possible aetiological agents in MS, a few are still being investigated.

Multiple sclerosis (MS) is the most common demyelinating disease of young adults, affecting over 250 000 individuals at any one time in the US alone. It is a chronic disease that begins in late adolescence or early adulthood. The pathological features of MS include the presence of demyelinating areas in the white matter of the brain, with perivascular inflammation and relative sparing of the axons. The distribution of plaques within the white matter is restricted to the CNS.

1. Background to the Viral Theory of the Pathogenesis of Multiple Sclerosis (MS)

The pathogenesis of MS is unknown, but it is currently accepted that a T cell-mediated autoimmune response is involved in the demyelinating process. Evidence for this hypothesis is found in epidemiological, genetic and immunological studies. The composition of CNS white matter infiltrates (consisting primarily of lymphocytes and monocytes), the association with genes that predispose to autoimmunity in human disease,^[1] the similarities

in both the symptoms and the physiological changes between MS and experimental, immune-mediated demyelinating disease, as well as the clinical response of symptoms of MS to immunosuppressive and immunomodulatory treatments, all support an autoimmune pathogenesis.

Recent studies suggest that environmental stimuli may have an important role in triggering autoimmunity in MS, possibly through mechanisms of molecular mimicry or superantigen production.^[2,3] Molecular mimicry indicates shared homologies between normal human myelin protein and viral polypeptides. An immune response against such a viral epitope can be extended to the homologue region of the normal human protein. In MS, homology between myelin antigens and viral peptides is established.^[4] Thus, this mechanism could result in CNS demyelination after viral infection. Superantigens are a class of immunostimulatory molecules produced by bacteria and viruses that can induce the activation of a large number of T cells.

The epidemiology of MS supports a geographi-

cal association with north-south gradients, with a higher prevalence in North America, Europe, Australia and New Zealand, and a lower prevalence in most of Asia, Africa and South America.^[5] A number of risk factors have been associated with susceptibility to MS (see table I).

Genetic factors play an important role in MS. Biological relatives of individuals with MS have a greater susceptibility to MS than adoptees, and the closer the biological relationship the greater the risk for developing MS. Although the rate of MS is 8 times higher in monozygotic than in dizygotic twins, genetics alone are insufficient to cause the disease. In identical twin pairs in whom one twin has MS, only 28% of the other twins develop the disease, even with long term follow-up. Despite sophisticated genetic studies, no single gene has been consistently linked to MS, although an association with the major histocompatibility complex (MHC) class II alleles DR2 and DQw1 has been demonstrated.^[6]

Clusters of MS outbreaks have also been reported, supporting a role for an infectious agent in the pathogenesis of this disease. The sudden appearance of MS on the Faroe Islands after the occupation by English troops in 1940 suggested an inter-human transmission of the disease.^[5,7] Rohowsky-Kochan et al.^[8] implicated canine distemper virus (CDV) as a possible causal agent of MS. The dog population of the Faroe Islands developed the neurologi-

cal form of CDV at the beginning of the war, which correlated with the introduction into the islands of guard dogs by the British troops. Other authors developed a tick-borne virus theory of MS,^[9] based on correlations of high rates of MS and specific tick species associated with migratory seabirds vectoring an arbovirus. Birds have also been considered to be carriers of viral vectors associated with a cluster of MS cases in Key West, Florida, US from 1972 to 1986.^[10]

In addition to the geographical association of disease susceptibility with evidence of MS, other data implicating a virus in the aetiology of MS include:^[11]

- Epidemiological evidence of childhood exposure to infectious agents in patients with MS.
- An increase in disease exacerbation with viral infection.
- Abnormal immune responses to a variety of viruses in patients with MS.
- Analogy with animal models and other human diseases in which viruses can cause disorders with long incubation periods, a relapsing-remitting course and demyelination.

Assuming MS is triggered by an infectious agent there are a variety of mechanisms by which demyelination may be induced:^[12]

- Viral infection of glial cells, such as oligodendrocytes, may directly damage these cells. However, as yet, no virus has been detected in the brain or other tissues of patients with MS. Despite this, recent examples of infectious diseases in which the agent was not identified for many years include *Helicobacter pylori*-induced peptic ulcer,^[13] *Borrelia burgdorferi* for Lyme disease^[14] and the cat scratch disease.^[15]
- Molecular mimicry may also play a role in MS where structural similarity between viral T cell epitopes and self-peptides could lead to the induction of an autoaggressive T cell response. Other examples of this mechanism may include post-measles encephalomyelitis and the Gullian-Barre syndrome.^[12]
- Immune activation by an MS-associated virus may lead to cytokine release and up-regulation of adhesion and MHC molecules, which in turn

Table I. Risk factors for multiple sclerosis^[4]

Risk factor	Characteristic
Race	White more than Black
Gender	Female : male (2 : 1)
Age	Between 25 and 55 years
Ethnicity	Caucasian
Latitude	Higher prevalence in extreme north and south of the globe
Weather conditions	High in temperate zones and in Western Europe, and low in tropical and subtropical areas
Migration	To and from high risk areas influences the likelihood of developing MS
Family history	
Human leucocyte antigen (HLA) haplotype	MS is associated with genes within or close to the HLA-DR-DQ subregion, e.g. HLA Dr15 in Caucasians

may activate myelin-specific T cells that may be associated with demyelination.

- A virus could cause recurrent infections resulting in exacerbation of MS. MS attacks could result from reactivation of a virus which is latent or persistent within the CNS [e.g. retrovirus, measles, herpes simplex virus (HSV)].

2. Other Demyelinating Diseases Associated with Viral Agents

Several viral agents have been found to be associated with demyelinating diseases. These findings strengthen the hypothesis that MS, also a demyelinating disease, may have a viral cause.

2.1 Animals

There are several animal viruses that can produce CNS demyelinating diseases, such as theiler murine encephalomyelitis virus (TMEV), semliki forest virus (SFV), mouse hepatitis (murine coronavirus-JHM) and lactic dehydrogenase virus (LDV).^[16] TMEV induces an acute infection with cell-mediated and humoral immune response resulting in apoptosis of infected grey matter cells and an acute poliomyelitis in affected mice. SFV and JHM are associated with a chronic immune response mediated by CD4⁺ cells. JHM virus induces an acute encephalomyelitis with scattered demyelinating lesions in mice and with necrotic lesions distributed throughout the CNS in rats. LDV may cause disease only in mice that encode a particular endogenous retroviral component.^[17]

Infections caused by Lentiviruses (a family of retroviruses, all of which produce chronic encephalopathies) are persistent and occur in large mammals such as cats, sheep, goats and horses. The most relevant examples for MS are the Visna-Maedi virus of sheep and the caprine arthritis-encephalomyelitis virus.^[16] Both viruses are associated with an active inflammatory process. The histopathology is similar to MS, with areas of demyelination in which the virus induces a strong cell immune-mediated response in infected animals.

CDV belongs to the morbilliviruses and is closely related to measles virus. Canine distemper is a wide-

spread disease that occurs in dogs and other members of the canine family. It can be acute or subacute and in rare cases it is followed by a demyelinating encephalomyelitis known as post-distemper encephalitis.^[16]

2.2 Humans

Progressive multifocal leukoencephalopathy (PML) is a rare white matter disease, which develops with multifocal symptoms. It occurs in conditions associated with defective cellular immunity. The human papovavirus JC is the causative agent of PML. This virus is widespread in the human population and associated with a subclinical infection. After a primary infection, the virus can be activated when the immune system is downregulated. Therefore, acquiring JC virus during a period of immunodeficiency may lead to the development of PML.^[16]

Subacute sclerosing panencephalitis is a rare disease of the CNS of children and young adults. It follows a natural measles virus infection after a variable period of latency. This demyelinating disease is caused by infection of the lymphoid organs and may lead to the activation of lymphocytes reactive against myelin basic protein (MBP) and other myelin constituents.^[18]

Human T-lymphotropic virus type I (HTLV-I)-associated myelopathy/tropical spastic paraparesis is a chronic progressive myelopathy with clinical similarities to MS. It is caused by infection with HTLV-I, a virus present throughout the world. It is an immunologically mediated disorder occurring in a small subset of genetically predisposed individuals.^[19]

Human immunodeficiency virus (HIV) encephalopathy and myelopathy are common clinical extensions of AIDS, in which the HIV infects cells within the CNS. Lesions in the white matter are probably the result of a direct HIV-cytopathic effect.^[20]

Post-infectious encephalomyelitis is a complication of an acute virus infection and is associated with measles virus, influenza virus, varicella-zoster virus and respiratory tract infections of viral cause.

This disease has also been reported after vaccinations against smallpox and rabies.^[18]

3. Viral Candidates in MS

Infectious agents have been postulated as a cause of MS for over a century;^[18] however, to date, no single virus has been definitively associated with this disease. Table II lists a number of viruses that, in the last 50 years, have been implicated in the aetiology of MS. Although most of these viruses are no longer considered as possible aetiological agents in MS, a few are still considered as candidate viruses.

Mechanistically, a virus in an immunologically susceptible host could induce the auto-destructive process observed in the CNS of patients with MS.^[43] Immunological recognition of viral peptides of sufficient structural similarity to the immunodominant MBP peptide may lead to clonal extension of MBP-reactive T cells in MS. Viruses such as HSV or Epstein Barr virus (EBV), which are known to cause latent or persistent infections, may under specific conditions, lead to a chronic antigenic stimulation of autoreactive T cell clones. An immunodominant region of MBP (84-102) is predominantly found in individuals who are carriers of HLA-DR2 and who have MS and has sequence homology to some viruses.^[2]

Retroviruses have long been considered as candidate agents in the pathogenesis of MS. In 1989, Perron et al.^[36] described the production of extracellular virions associated with reverse transcriptase activity by a culture of leptomenigeal cells (LM7) obtained from the CSF of a patient with MS. More recently, the same group was able to identify a specific retroviral sequence associated with virions produced by cell cultures from several patients with MS.^[42] They called this new retrovirus MS-associated retrovirus (MSRV). Its molecular characterisation has revealed that it is distantly related (75% homology) to the sequence of ERV9 (a well characterised endogenous retrovirus), but at present it is still unclear whether MSRV itself is exogenous or endogenous. However, Brahic and Bureau^[44] have suggested that sequences nearly identical to

MSRV have been described previously, are endogenous and are expressed both in MS and control human tissues.

Recently, the newly discovered human herpes virus 6 (HHV-6)^[45] has been reported to be a potential viral candidate in MS. This T-lymphotropic β -herpes virus has been identified as the aetiological agent for exanthema subitum (roseola),^[46] but CNS complications occur frequently during the course of HHV-6-associated disease. In addition, HHV-6 has been associated with encephalitis, encephalopathy^[47-49] and myelopathy,^[50] with possible latent infection in the CNS.^[51] Recently, He et al.^[52] showed that HHV-6 can infect primary fetal astrocytes *in vitro* and that the progeny virus could reinfect T cells. These findings suggest that infection of primary human astrocytes may play a role in the neuropathogenesis of HHV-6.

In the last few years the possible role of HHV-6 in MS has been investigated. Different groups have shown correlations between a possible reactivation of the virus and the onset or development of MS. Sola et al.,^[41] using an immunofluorescence assay found significantly higher anti-HHV-6 immunoglobulin (Ig) G titres in the serum and CSF of patients with MS in comparison with a normal donor group. In 1995, Challoner et al.,^[40] using representational difference analysis, detected HHV-6 DNA in 70% of brain samples from individuals with MS and controls, suggesting that the HHV-6 is a common commensal virus of the brain. In addition, monoclonal antibodies against HHV-6 had detected the virus only in brain tissues of MS patients and not controls. More recently, Soldan et al.^[11] demonstrated increased IgM serum antibody responses to HHV-6 early antigen in patients with relapsing-remitting MS, compared with patients with chronic progressive MS, patients with other neurological and autoimmune disease, and healthy controls.

These findings were supported by the detection of HHV-6 cell-free viral DNA in the serum of 30% of patients with MS compared with 0% in controls, using a nested polymerase chain reaction technique.^[53] However, other studies have failed to detect HHV-6 DNA in CSF and peripheral blood mono-

Table II. Viruses postulated to be the cause of multiple sclerosis (MS)^[18]

Virus	Evidence of association	Year described
Rabies virus	Isolated from blood and CSF of patients with MS	1946 ^[21] , 1964 ^[22]
Scrapie agent	Scrapie developed in sheep after inoculation with samples from the brain of a patient who had had MS	1962 ^[23] , 1965
HSV	Isolated in T cells from brain samples of patients who had had MS Increased CSF antibody titer	1964 ^[24] 1978 ^[25]
Multiple sclerosis-associated agent	Decrease in polymorphonuclear cells in mice inoculated with tissue from a patient who had had MS	1972 ^[26]
Parainfluenza virus	Isolated in T cells after fusion of brain cells from 2 patients with MS	1972 ^[27]
Measles virus	Development of cytopathic effect in T cell after inoculation with samples from the brain of a patient who had had MS	1972 ^[28] , 1989 ^[29]
Simian virus 5	Development of cytopathic effect in T cell after inoculation with BM from a patient who had had MS	1978 ^[30]
Cytomegalovirus	Isolated from a chimpanzee inoculated with brain cells from a patient who had had MS	1979 ^[31]
Coronavirus	Isolated from mice inoculated with samples from the brain of a patient who had had MS	1980 ^[32]
SMON-like virus	Isolated from the CSF of a patient with MS	1982 ^[33]
Tick-borne encephalitis flavivirus	Isolated from mice inoculated with blood from a patient with MS	1982 ^[34]
HTLV-I	Detection of retrovirus in T cells from CSF of patients with MS	1985 ^[35]
Leptomeningeal cells (LM7)	Isolated from leptomeningeal cell line obtained from the CSF of a patient with MS	1989 ^[36]
HSV-I	Virus isolated from the CSF of a patient with MS	1989 ^[37]
Herpes and retrovirus	Herpes simplex/EBV transactivation of retrovirus	1993 ^[38] , 1997 ^[39]
HHV-6	Detection of DNA and proteins in tissue from a patient with MS Detection of antibodies and DNA in sera from a patient with MS	1995 ^[40] 1993 ^[41] , 1997 ^[11]
MSRV	Detection of retroviral RNA in the CSF of patients with MS	1997 ^[42]

BM = bone marrow; **EBV** = Epstein Barr virus; **HHV** = human herpes virus; **HSV** = herpes simplex virus; **HTLV** = human T-lymphocytic virus; **MSRV** = MS-associated retrovirus; **SMON** = subacute myelo-optico neuropathy.

nuclear cells of patients with MS, showing a lack of correlation between HHV-6 infection and the development of MS.^[54,55]

The hypothesis that DNA viruses such as herpes viruses can transactivate retroviruses has also been reported.^[38,39] One of the first pieces of evidence for interaction between herpes virus and retrovirus is proposed in a study of an avian model of Burkitt's lymphoma.^[56] Reactivation of retroviruses by superinfection with another virus is a common phenomenon and has also been observed with members of the herpes virus group or hepatitis B virus in HIV infection.^[57] More recently Sommerlund et al.^[58] established a continuously growing B cell line producing retrovirus-like particles and EBV from a patient with a chronic progressive myelopathy resembling MS.

4. Therapeutic Implications of a Viral Cause of MS

The aetiology and pathogenesis of MS are still unclear. While no cure is available for this disease, it is no longer considered untreatable. For the past few decades, corticosteroids have been considered the treatment of choice for exacerbations of MS. Their anti-inflammatory and immunosuppressive effects rapidly block the inflammatory stage of the lesion and lead to closing of the blood-brain barrier. The result is to shorten the duration and severity of exacerbations. However, corticosteroids have no effect on the long term course of the disease and cannot be given continuously because of adverse effects.

Recently, a number of therapeutic strategies have been demonstrated to be effective in MS. Interferon-

β (IFN β) is a cytokine secreted upon viral infection by a range of cells including lymphocytes macrophages and dendritic cells. In addition to inhibiting viral spread it counteracts many of the immunostimulatory effects of interferon- γ (IFN γ). In regard to this review and the role of viruses in MS, clinical trials using interferons (both α and β) have been shown to be effective in relapsing-remitting MS,^[59-61] preventing both exacerbation and new lesions [as detected by magnetic resonance imaging (MRI)].

In our laboratory we have begun to correlate the presence of serum HHV-6 DNA of patients with MS with MRI analysis of MS disease activity and the effect of immunotherapeutic treatment of this disorder. Recently, aciclovir (acyclovir), a drug that selectively inhibits replication of herpes viruses, has been used to test the hypothesis that herpes virus infections are involved in the pathogenesis of relapsing-remitting MS.^[61] The use of this drug decreased exacerbations by 34%, suggesting that acyclovir-susceptible viruses might be involved in the pathogenesis of MS, in particular in the induction of exacerbations. It is clear that a better understanding of the characteristics of pathological agent(s) would lead to the development of appropriate therapeutic strategies for MS.

5. Conclusion

Even if the aetiology of MS is still unsolved, many lines of evidence suggest that a viral infection during childhood, in association with an immune response, induces MS. An infectious agent has been postulated as the cause of MS for over a century and caution must be taken in the interpretation of studies suggesting viral associations in this disorder. However, even if no virus has been definitively implicated in the aetiology of MS, the failure to demonstrate a virus in this disease does not mean that it is not present: 'absence of evidence is not evidence of absence'.^[62]

References

1. Vyse TJ, Todd JA. Genetic analysis of autoimmune disease. *Cell* 1996; 85 (3): 311-8
2. Wucherpfening KW, Strominger JL. Molecular mimicry in T-cell mediated autoimmunity: viral peptides activated human

- T cell clones specific for myelin basic protein. *Cell* 1995; 80: 695-705
3. Marrack P, Kappler J. Subversion of the immune system by pathogens. *Cell* 1994; 76: 323-32
4. Lynch SG, Rose JW. Multiple sclerosis. *Dis Mon* 1996; 42 (1): 1-55
5. Kurtzke JF. MS epidemiology world wide. One view of current status. *Acta Neurol Scand* 1995; Suppl. 161: 23-33
6. Cook SD. Multiple Sclerosis and viruses. *Mult Scler* 1997; 3 (6): 388-9
7. Kurtzke JF, Hyllested K, Heltberg A. Multiple sclerosis in the Faroe Islands: transmission across four epidemics. *Acta Neurol Scand* 1995; 91: 321-5
8. Rohowsky-Kochan C, Dowling PC, Cook SD. Canine distemper virus-specific antibodies in multiple sclerosis. *Neurology* 1995; 45 (8): 1554-60
9. Brown JS Jr. Geographic correlation of multiple sclerosis with tick-borne diseases. *Mult Scler* 1996; 1: 257-61
10. MacGregor HS, Latiwonk QI. Search for the origin of multiple sclerosis by first identifying the vector. *Med Hypothesis* 1992; 37: 67-73
11. Soldan SS, Berti R, Salem N. Association of human herpes virus 6 (HHV-6) with multiple sclerosis: increased IgM response to HHV-6 early antigen and detection of serum HHV-6 DNA. *Nature Med* 1997 Dec; 3 (12): 1394-7
12. Cook SD, Rohowsky-Kochan C, Bansil S. Evidence for multiple sclerosis as an infectious disease. *Acta Neurol Scand* 1995; Suppl. 161: 34-42
13. Tytgat GN. *Helicobacter pylori*: causal agent in peptic ulcer disease? Conclusion. *J Gastroenterol Hepatol* 1991; 6 (2): 139-40
14. Pachner AR, Duray P, Steere AC. Central nervous system manifestations of Lyme disease. *Arch Neurol* 1989 Jul; 46 (7): 790-5
15. Gerber MA, Sedgwick AK, MacAlister TJ. The aetiological agent of cat scratch disease. *Lancet* 1985 Jun 1; 1 (8440): 1236-9
16. Volker ter Meulen V, Katz M. The proposed viral aetiology of multiple sclerosis and related demyelinating diseases. In: Raine CS, Mc Farland HF, Tourtellotte WW, editors. Multiple sclerosis clinical and pathogenetic basis. Cambridge: Chapman & Hall, 1997: 287-305
17. Pease LR, Murphy WH. Co-infection by lactic dehydrogenas virus and C-type retrovirus elicits neurological disease. *Nature* 1980; 286: 398-400
18. Johnson RT. The virology of demyelinating diseases. *Ann Neurol* 1994; 36: S54-60
19. Osame M, Usuku K, Izumo S, et al. HTLV I associated myelopathy, a new clinical entity. *Lancet* 1986; II: 1031-2
20. Rice GP. Virus-induced demyelination in man: models for multiple sclerosis. *Curr Opin Neurol Neurosurg* 1992; 5 (2): 188-94
21. Margulis MS, Soloviev VD, Shubladze AK. Aetiology and pathogenesis of acute sporadic disseminated encephalomyelitis and multiple sclerosis. *J Neurol Neurosurg Psychiatry* 1946; 9: 63-74
22. Bychkova EN. Viruses isolated from patients with encephalomyelitis and multiple sclerosis. I. Pathogenetic and antigenic properties. *Fed Proc Transl* 1965; Suppl. 24 (4): 742-4
23. Field EJ, Miller H, Russel DS. Observation of glial inclusion bodies in case of acute disseminated sclerosis. *J Clin Pathol* 1962; 15: 278-84
24. Gudnadottir M, Helgadottir H, Bjarnason O, et al. Virus isolated from the brain of a patient with multiple sclerosis. *Exp Neurol* 1964; 9: 85-95
25. Norrby E. Viral antibodies in multiple sclerosis. *Prog Med Virol* 1978; 24: 1-39
26. Carp RI, Licursi PC, Merz PA, et al. Decrease percentages of polymorphonuclear neutrophils in mouse peripheral blood af-

- ter inoculation with material from multiple sclerosis patients. *J Exp Med* 1972; 136: 618-29
27. ter Meulen V, Koprowski H, Iwasaki Y, et al. Fusion of cultured multiple-sclerosis brain cells with indicator cells: presence of nucleocapsid and virion and isolation of parainfluenza-type virus. *Lancet* 1972; I: 1-5
 28. Field EJ, Cowshall S, Narang HK, et al. Viruses in multiple sclerosis? *Lancet* 1972; II: 280-1
 29. Cosby SL, McQuaid S, Taylor MJ, et al. Examination of eight cases of multiple sclerosis and 56 neurological and non-neurological controls for genomic sequences of measles virus, canine distemper virus, simian virus 5 and rubella virus. *J Gen Virol* 1989; 70: 2027-36
 30. Mitchell DN, Porterfield JS, Micheletti R, et al. Isolation of an infectious agent from bone-marrow of patients with multiple sclerosis. *Lancet* 1978; II: 387-90
 31. Wroblecka Z, Gilden D, Devlin M, et al. Cytomegalovirus isolation from a chimpanzee with acute demyelinating disease after inoculation of multiple sclerosis brain cells. *Infect Immun* 1979; 25: 1008-15
 32. Burks JS, Devald BL, Jankovsky LD, et al. Two coronaviruses isolated from central nervous system tissue of two multiple sclerosis patients. *Science* 1980; 209: 933-4
 33. Melnick JL, Seidel E, Inoue YK, et al. Isolation of virus from the spinal fluid of three patients with multiple sclerosis and one with amyotrophic lateral sclerosis. *Lancet* 1982; I: 830-3
 34. Vagabov RMA, Skortsova TM, Gofman YP, et al. Isolation of the tick-borne encephalitis virus from a patient with multiple sclerosis. *Acta Virol* 1982; 26 (5): 403
 35. Koprowski H, DeFreitas EC, Harper ME, et al. Multiple sclerosis and human T cell lymphotropic retroviruses. *Nature* 1985; 318: 154-60
 36. Perron H, Geny C, Laurent A, et al. Leptomeningeal cell line from multiple sclerosis with reverse transcriptase activity and viral particles. *Res Virol* 1989; 140: 551-61
 37. Bergstrom T, Andersen O, Vahne A. Isolation of herpes simplex virus type 1 during first attack of multiple sclerosis. *Ann Neurol* 1989; 26: 283-5
 38. Perron H, Suh M, Lalande B, et al. Herpes simplex virus ICPO and ICP4 immediate early proteins strongly enhance expression of a retrovirus harbored by a leptomeningeal cell line from a patient with multiple sclerosis. *J Gen Virol* 1993; 74: 65-72
 39. Munch M, Hvas J, Christensen T, et al. The implication of Epstein-Barr virus in multiple sclerosis – a review. *Acta Neurol Scand* 1997; Suppl 169: 59-64
 40. Challoner PB, Smith KT, Parker JD, et al. Plaque-associated expression of human herpes virus 6 in multiple sclerosis. *Proc Natl Acad Sci U S A* 1995; 92: 7440-4
 41. Sola P, Merelli E, Marasca R, et al. Human herpesvirus 6 and multiple sclerosis: survey of anti-HHV-6 antibodies by immunofluorescence analysis and of viral sequences by polymerase chain reaction. *J Neurol Neurosurg Psychiatry* 1993; 56: 917-9
 42. Perron H, Garson JA, Bedin F, et al. Molecular identification of a novel retrovirus repeatedly isolated from patients with multiple sclerosis. *Proc Natl Acad Sci U S A* 1997; 94: 7583-8
 43. Dalgleish AG. Viruses and multiple sclerosis. *Acta Neurol Scand* 1997; Suppl. 169: 8-15
 44. Brahic M, Bureau JF. Multiple sclerosis and retroviruses. *Ann Neurol* 1997; 42: 984-5
 45. Salahuddin SZ, Ablashi DV, Markham PD, et al. Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. *Science* 1986; 234 (4776): 596-601
 46. Yamanishi K, Okuno T, Shiraki M, et al. Identification of human herpesvirus-6 as a causal agent for exanthem subitum. *Lancet* 1988; I: 1065-7
 47. Drobyski WR, Knox KK, Majewski D, et al. Fatal encephalitis due to variant B human herpesvirus-6 infection in a bone marrow-transplant recipient. *N Engl J Med* 1994; 330 (19): 1356-60
 48. Knox KK, Carrigan DR. Active human herpesvirus (HHV-6) infection of the central nervous system in patients with AIDS. *J Acquir Immune Defic Syndr Hum Retrovirol* 1995; 9: 69-73
 49. Knox KK, Harrington DP, Carrigan DR. Fulminant human herpesvirus six encephalitis in a human immunodeficiency virus-infected infant. *J Med Virol* 1995; 45: 288-92
 50. Mackenzie IRA, Carrigan DR, Wiley CA. Chronic myelopathy associated with human herpesvirus-6. *Neurology* 1995; 45: 2015-7
 51. Caserta MT, Hall CB, Schnabel K, et al. Neuroinvasion and persistence of human herpesvirus 6 in children. *J Infect Dis* 1994; 170: 1586-9
 52. He J, McCarthy M, Zhou Y, et al. Infection of primary human fetal astrocytes by human herpesvirus 6. *J Virol* Feb 1996; 1296-300
 53. Secchiero P, Carrigan DR, Asano Y, et al. Detection of human herpesvirus 6 in plasma of children with primary infection and immunosuppressed patients by polymerase chain reaction. *J Infect Disease* 1995; 171: 273-80
 54. Martin C, Enbom M, Soderstrom M, et al. Absence of seven human herpesviruses, including HHV-6, by polymerase chain reaction in CSF and blood from patients with multiple sclerosis. *Acta Neurol Scand* 1997 May; 95 (5): 280-3
 55. Mayne M, Krishnan J, Metz L, et al. Infrequent detection of human herpesvirus 6 DNA in peripheral blood mononuclear cells from multiple sclerosis patients. *Ann Neurol* Sept 1998; 44 (3): 391-4
 56. Peters WP, Kufe D, Schlom J, et al. Biological and biochemical evidence for an interaction between Marek's disease herpesvirus and avian leukemia virus *in vivo*. *Proc Natl Acad Sci U S A* 1973; 70: 3175-8
 57. Chinnadurai G. Modulation of HIV-enhancer activity by heterologous agents: a mini review. *Gene* 1991; 101: 165-70
 58. Sommerlund M, Pallesen G, Moller-Larsen A, et al. Retrovirus-like particles in an Epstein-Barr virus producing cell line derived from a patient with chronic progressive myelopathy. *Acta Neurol Scand* 1993; 87: 71-6
 59. IFNB Multiple Sclerosis Study Group. Interferon beta 1-b is effective in relapsing-remitting multiple sclerosis. *Neurology* 1993; 43: 655-61
 60. Durelli L, Bongioanni MR, Cavallo R, et al. Chronic systemic high-dose recombinant interferon alfa-2a reduces exacerbation rate, MRI signs of disease activity, and lymphocyte interferon gamma production in relapsing-remitting multiple sclerosis. *Neurology* 1994; 44: 406-13
 61. Lycke J, Svennerholm B, Hjelmqvist, et al. Acyclovir treatment of relapsing-remitting multiple sclerosis. *J Neurol* 1996; 243: 214-24
 62. Mims CA. Viral aetiology of diseases of obscure origin. *Br Med Bull* 1985; 41: 63-9

Correspondence and reprints: Dr Steven Jacobson, Viral Immunology Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Building 10, Room 5-B16, 9000 Rockville Pike, Bethesda, MD 20892, USA.
E-mail: stevej@helix.nih.gov