Journal of NeuroVirology (2000) 6, Suppl 2, S76–S79

www.jneurovirol.com

The association between multiple sclerosis and infection with Epstein-Barr virus and retrovirus

Sven Haahr*[,1] and Mette Munch[1]

[1]*Department of Medical Microbiology and Immunology, The Bartholin Building, University of Aarhus, DK-8000 Aarhus C, Denmark*

B-lymphoblastoid cell-lines may develop spontaneously in mononuclear cells from patients with multiple sclerosis, an observation rarely seen in healthy individuals. Examination of such spontaneously established B-cell lines reveal the presence of Epstein-Barr virus and retrovirus particles. We have speculated that in predisposed individuals, a dual infection with retrovirus and late acquired Epstein-Barr virus plays an aetiological role in the development of multiple sclerosis. This hypothesis is supported by a number of observations, including the finding that infection with Epstein-Barr virus may be a prerequisite for developing multiple sclerosis. The association between multiple sclerosis and infection with Epstein-Barr virus and retrovirus is evaluated in this study. *Journal of NeuroVirology* (2000) **6,** S76–S79.

Keywords: multiple sclerosis; endogenous retrovirus; dual infection; interaction; B-lymphoblastoid cell-line; epidemiology

An infectious aetiology of multiple sclerosis (MS) has been suggested (Marié, 1887) since as early as the first scientific description of the disease which was presented more than 130 years ago (Charcot, 1868). Since this suggestion, a number of environmental agents, most often virus, have been association with MS, but only inconclusive and unconfirmed findings exist for such an association. The initial, and still the most convincing suggestion, that infectious agents are involved in MS originates from epidemiological studies (Martyn, 1991; Kurtzke, 1993; Gale and Martyn, 1995). These studies, and recent studies in monozygotic twins, point toward (an) environmental agent(s) which play an essential role in development of MS (Sawcer *et al*, 1997).

In 1985 the human retrovirus HTLV-I was associated with the chronic progressive myelopathy called HAM/TSP (Gessain *et al*, 1985), which in many ways resembles MS. At the same time, claims of an association between MS and retrovirus were published (Koprowski *et al*, 1985). Subsequent studies, however, were unable to confirm such an association (Bangham *et al*, 1989; Richardson *et al*, 1989).

*Correspondence: S Haahr

In further studies on the possible association between MS and retrovirus, retrovirus-like structures were observed by transmission electron microscopy on two occasions in T-cell clones from an MS patient (Haahr *et al*, 1991a). Later our group established spontaneously developing B-lymphoblastoid cell lines from a patient with an MS-like disease (Sommerlund *et al*, 1993). It was subsequently confirmed that these cell lines were more likely to develop from MS patients than from healthy controls (Munch *et al*, 1995; Fraser *et al*, 1979). These cell lines demonstrated co-expression of Epstein-Barr virus (EBV) and a C-type like retrovirus (Sommerlund *et al*, 1993; Munch *et al*, 1995).

A hypothesis was put forward by our group that MS is caused by a dual infection with retrovirus and late infection with EBV (Haahr *et al*, 1992). In particular this hypothesis is attractive because a mutual transactivation between viruses from the herpes group and the retrovirus group is known (Evermann *et al*, 1991).

A French group had previously described production of retrovirus in a leptomeningeal cell line obtained from a patient with MS (Perron *et al*, 1989), later in macrophages from MS patients (Perron *et al*, 1991) and subsequently in EBV-immortalized B cells from MS patients (Perron *et al*, 1997). However, based on epidemiological

studies, it had been argued that MS is not caused by a C-type-like retrovirus (Haahr *et al*, 1991b; Martyn, 1991), a view previously expressed (Operskalski *et al*, 1989).

Because the relapsing-remitting phase of MS in many ways is analogous to the recurrence of herpes virus infections, a virus from this group has been an attractive aetiologic candidate to MS. An association between MS and infection with EBV had previously been suggested (Warner and Carp, 1988).

Most studies have shown a 100% seropositivity in MS patients, in contrast to healthy controls who express a significantly lower seropositivity (Sumaya *et al*, 1980, 1985; Bray *et al*, 1983; Larsen *et al*, 1985). A single study however indicated the same seropositivity in MS patients and controls, most likely because of a low sensitivity of the serologic test used (Compston *et al*, 1986). Indeed using sensitive and specific tests, only one MS patient out of 478 has been found seronegative (Larsen *et al*, 1985; Sumaya *et al*, 1985; Munch *et al*, 1998a; Myhr *et al*, 1998). This serum sample originated from a deceased person. Therefore it was not possible to verify whether a careless handling of the specimen can explain the negative test result or whether negative EBV serology can occur in an MS patient (Munch *et al*, 1998). In one study, a significantly higher number of healthy controls with primary EBV infections were observed, suggesting a lack of primary infections in MS patients (Munch *et al*, 1998a). However, demyelinating disease after neurologically complicated primary EBV infections has been reported (Shaw *et al*, 1987; Bray *et al*, 1992).

A case-control study has demonstrated a significantly higher previous occurrence of infectious mononucleosis among MS patients. This suggests an older age at exposure to EBV in MS patients compared with healthy controls (Operskalski *et al*, 1989). We therefore performed an historical prospective study. We searched nearly 7000 diagnosed infectious mononucleosis (IM) patients in the nationwide Danish MS Registry and compared them with nearly 13 000 patients with an IM-like disease with a negative IM diagnosis. The patients with a positive IM diagnosis had a 2.8 times higher risk of developing MS than those with a negative IM diagnosis. Importantly, none of the patients with a positive IM diagnosis had developed MS before the diagnostic test. In contrast, ten patients had developed MS among those with a negative test (Haahr *et al*, 1995).

Occurrence of clusters of MS patients has often been considered an indication of an infectious aetiology of MS, although none of these studies have searched for specific etiologic agents (Riise, 1997). Recently, we reported clusters of MS patients in Denmark (Haahr *et al*, 1998). EBV subtyping was performed on members of two clusters comprising three and eight patients. The same subtype of EBV was observed in members of these two clusters out of six various subtypes observed in the study. Although a preponderance of this subtype was found both in MS patients and healthy controls, the presence of this subtype in all studied cluster members is significantly different from the finding in the healthy control group (n=16), which included eight schoolmates to the cluster members and eight randomly selected healthy persons. The observation of only one subtype in the MS-clusters is also significantly different from that observed in all non-clustered individuals studied (n=44) (Munch *et al*, 1998b). An involvement of EBV in MS may explain why MS patients within the same birth cohort have lived in closer proximity between 13 and 20 years of age than would be expected (Riise *et al*, 1991). The migration studies, which are the most substantiated epidemiological investigations, clearly show that the risk of MS may change after migration between places with variable prevalence of the disease. Importantly, this may imply that MS is preventable (Norrby, 1978). An agent which, in predisposed persons, may initiate MS when infecting after puberty, but protecting against MS, when infecting before puberty, would be able to explain the migration studies in MS. EBV is suggested to be a serious candidate agent (Gale and Martyn, 1995).

The retroviruses observed by the French and the Danish research groups (Perron *et al*, 1989; Sommerlund *et al*, 1993; Munch *et al*, 1995) have now been further characterized. Partial molecular characterization by the French group in collaboration with an English research group revealed that the retrovirus was related (about 75% homology) to the endogenous retrovirus ERV9 (Perron *et al*, 1997). They detected virus RNA in circulating virions from serum obtained from MS patients (nine of 17)– especially in serum from untreated MS patients (six of six) and only exceptionally in non-MS controls (three of 44) (Garson *et al*, 1998). A Danish research group (Christensen *et al*, 1998) in collaboration with the Institute of Biotechnology, Cophenhagen, has characterized virus from four spontaneously developed B-lymphoblastoid cell-lines (Sommerlund *et al*, 1993; Munch *et al*, 1995). In several *gag* and *env* fragments, homology to the human endogenous retrovirus RGH-2 has been identified. Expression of RGH-2 sequences at particle levels was detected in plasma from 22 of 31 MS patients. Sequences from the endogenous RGH-2 retrovirus were expressed in plasma from all MS patients with active disease (n=9) at the time of sampling, where all healthy controls (n=18) and all patients with non-neurological diseases (n=29) were negative (Christensen *et al*, 1998).

Although it has not been rigorously demonstrated that infection with EBV is a prerequisite for developing MS, circumstantial evidence points toward this possibility. Additional studies support the notion that persons with late EBV infection have a higher risk for developing MS (Lindberg *et al*,

1991; Martyn *et al*, 1993). If EBV can be shown to be involved in the pathogenesis of MS, the possibility for developing a drug influencing the multiplication of EBV may be within reach, and in this way it may be possible to influence progression of MS. Moreover, prevention of MS may be possible through development of a safe and efficient vaccine against EBV. The consequence(s) of the expression of the endogenous retrovirus are still unknown. Are they expressed because of the disease or are they contributing to the disease process? Studies on the transmissibility of these endogenous retroviruses *in vivo* need to be known before any speculation about their possible role in the epidemiology of MS.

Acknowledgements

The studies referred in this review were funded by grants from the Danish MS Society, The John and Birthe Meyer Foundation, and The Johnsen and Wife Foundation. We thank Margit Schjerven, Ruth Nielsen and Margit Aagaard for excellent technical assistance and Tove Christensen and Anné Moller-Larsen for valuable contributions to this study.

References

Bangham CRM, Nightingale S, Cruickshang JK, Daenke S (1989). PCR analysis of DNA from multiple sclerosis patients for the presence of HTLV-I. *Science* **246:** 821.

Bray PF, Bloomer LC, Salmon VC, Bagley MH, Larsen PD (1983). Epstein-Barr virus infection and antibody synthesis in patients with multiple sclerosis. *Arch Neurol* **40:** 406–408.

Bray PF, Culp KW, McFarlin DE, Panitch HS, Torkelsen RD, Schlight JP (1992). Demyelinating disease after neurologically complicated primary Epstein-Barr virus infection. *Neurology* **42:** 278–282.

Charcot JM (1868). Histologie de la sclerose en plaque. *Gaz Hosp (Paris)* **41:** 551–554.

Christensen T, Dissing Sorensen P, Riemann H, Hansen HJ, Møller-Larsen A (1998). Expression of sequence variants of endogenous retrovirus RGH in particle form in multiple sclerosis. *Lancet* **352:** 1033.

Compston DAS, Vakarelis BN, Paul E, McDonald WI, Batchelor JR, Mims CA (1986). Viral infection in patients with multiple sclerosis and HLA-DR matched controls. *Brain* **109:** 325–344.

Evermann JF, Derse D, Dern PL (1991). Interactions between herpesvirus and retroviruses: implications in the initiation of disease. *Microbial Pathogenesis* **10:** 1–9.

Fraser KB, Haire M, Millar JHD, McCrea S (1979). Increased tendency to spontaneous in-vitro lymphocyte transformation in clinically active multiple sclerosis. *Lancet* **ii:** 715–717.

Gale CR, Martyn CN (1995). Migrant studies in multiple sclerosis. *Prog Neurobiol* **47:** 425–448.

Garson JA, Tuke PW, Giraud P, Paranhoe-Buccala G, Perron H (1998). Detection of virion-associated MSRV-RNA in serum of patients with multiple sclerosis. *Lancet* **351:** 33.

Gessain A, Barin F, Vernant JC, Gout O, Maurs L, Calendar A, de Thé G (1985). Antibodies to HTLV-I in patients with tropical spastic paraparesis. *Lancet* **ii:** 407–410.

Haahr S, Sommerlund M, Nielsen R, Møller-Larsen A, Hansen HJ (1991a). Just another dubious virus in cells from a patient with multiple sclerosis. *Lancet* **i:** 663–664.

Haahr S, Sommerlund M, Møller-Larsen A (1991b). Multiple sclerosis as a retroviral disease? Epidemiological consideration in relation to HTLV-I epidemiology. *Neuroepidemiology* **10:** 232–235.

Haahr S, Sommerlund M, Møller-Larsen A, Mogensen S, Andersen HMK (1992). Is multiple sclerosis caused by a dual infection with retrovirus and Epstein-Barr virus? *Neuroepidemiology* **11:** 299–303.

Haahr S, Koch-Henriksen N, Møller-Larsen A, Eriksen LS, Andersen HMK (1995). Increased risk of multiple sclerosis after late Epstein-Barr virus infection: a historical prospective study. *Multiple Sclerosis* **1:** 73–77.

Haahr S, Munch M, Christensen T, Møller-Larsen A, Hvas J (1998). Cluster of multiple sclerosis patients from Danish community. *Lancet* **349:** 923.

Koprowski H, deFreitas EC, Harper ME, Sandberg-Wollheim M, Sheremata WA, Robert-Guroff M, Saxinger CW, Feinberg MB, Womg-Staal F, Gallo RC (1985). Multiple sclerosis and human T-cell lymphotropic retroviruses. *Nature* **318:** 154–160.

Kurtzke JF (1993). Epidemiological evidence for multiple sclerosis as an infection. *Clin Microbiol Rev* **6:** 382–427.

Larsen PD, Bloomer LC, Bray PF (1985). Epstein-Barr virus nuclear antigen and viral capsid antigen antibody titers in multiple sclersosis. *Neurology* **35:** 435–438.

Lindberg C, Andersen O, Vahlne A, Dalton M, Rumaker B (1991). Epidemiological investigations of the association between infectious mononucleosis and multiple sclerosis. *Neuroepidemiology* **10:** 62–65.

Marié P. (1884). Sclérose en plaques et maladies infectueuses. *Prog Med* **12:** 287–289.

Martyn CN (1991). The epidemiology of multiple sclerosis. In: *McAlpin's Multiple Sclerosis*. Matthews WB (ed). Churchill Livingston: Edinburgh, London, Melbourne and New York. pp 3–40.

Martyn CN, Cruddas M, Compston DAS (1993). Symptomatik Epstein-Barr virus infection and multiple sclerosis. *J Neurol Neurosurg Psych* **56:** 167–168.

Munch M, Møller-Larsen A, Christensen T, Morling N, Hansen HJ, Haahr S (1995). B-lymphoblastoid cell-lines from multiple sclerosis patients and a healthy control producing a putative new human retrovirus and Epstein-Barr virus. *Multiple Sclerosis* **1:** 78–81.

Munch M, Riisom K, Christensen, Møller-Larsen A, Haahr S (1998a). The significance of Epstein-Barr virus seropositivity in multiple sclerosis patients? *Acta Neurol Scand* **97:** 171–174.

Munch M, Hvas J, Christensen T, Møller-Larsen A, Haahr S (1998b). A single subtype of Epstein-Barr virus in members of multiple sclerosis clusters. *Acta Neurol Scand* **98:** 395–399.

Myhr KM, Riise T, Barren-Connor E, Myrmel H, Vedeler C, Grønning M, Kalvenes MB, Nyland H (1998). Altered antibody pattern to Epstein-Barr virus but not to other herpesviruses in multiple sclerosis: a population based case-control study from western Norway. *J Neurol Neurosurg Psychiatry* **64:** 539–542.

Norrby E (1978). Viral antibodies in multiple sclerosis. *Prog Med Virol* **24:** 1–39.

Operskalski EA, Visscher BR, Malmgren RM, Detels R (1989). A case-control study of multiple sclerosis. *Neurology* **39:** 825–839.

Perron H, Geny C, Laurent A, Mouriquand C, Pellet I, Perret J, Seigneurin JM (1989). Leptomeningeal cell line from multiple sclerosis with reverse transcriptase activity and viral particles. *Res Virol* **140:** 551–561.

Perron H, Lalande B, Gratacap B, Laurent A, Geny C, Mallaret M, Schuller E, Stroebner P, Seigneurin JM (1991). Isolation of retrovirus from patients with multiple sclerosis. *Lancet* **337:** 862–863.

Perron H, Garson JA, Bedin F, Beseme F, Paranhos-Baccala G, Komurian-Pradel F, Mallet F, Tuke PW, Voisset C, Blond JL, Lalande B, Seigneurin JM, Mandrand B and The Collaborative Research Group on Multiple Sclerosis (1997). Molecular identification of a novel retrovirus repeatedly isolated from patients with multiple sclerosis. *Proc Natl Acad Sci* **94:** 7583–7588.

Richardson JH, Wucherpfenning KW, Ende N, Rudge P, Dalgleish AG, Hafler DA (1989). PCR analysis of DNA from multiple sclerosis patients for the presence of HTLV-I. *Science* **246:** 821–823.

Riise T, Grønning M, Klauber MR, Barrett-Connor E, Nyland H, Albrectsen G (1991). Clustering of residence of multiple sclerosis patients at age 13–20 years in Hordeland, Norway. *Am J Epidemiol* **133:** 932–939.

Riise T (1997). Cluster studies in multiple sclerosis. *Neurology* **49** (suppl 2): S27–S32.

Sawcer S, Robertson N, Compston (1997). Genetic epidemiology of multiple sclerosis. In: *Multiple Sclerosis: Clinical Challenges and Controversies*. Thompson AL, Polman C, Hohlfeld R (eds). Martin Dunitz, London. pp 13–29.

Shaw CM, Alvord Jr EC (1987). Multiple sclerosis beginning in infancy. *J Child Neurol* **2:** 252–256.

Sommerlund M, Pallesen G, Møller-Larsen A, Hansen HJ, Haahr S (1993). Retrovirus-like particles in an Epstein-Barr virus producing cell line derived from a patient with chronic progressive myelopathy. *Acta Neurol Scand* **87:** 71–76.

Sumaya CV, Myers LW, Ellison GW (1980). Epstein-Barr virus antibodies in multiple sclerosis. *Arch Neurol* **37:** 94–96.

Sumaya CV, Myers LW, Ellison GW, Ench Y (1985). Increased prevalence and titer of Epstein-Barr virus antibodies in patients with multiple sclerosis. *Ann Neurol* **17:** 371–377.

Warner HB, Carp RI (1988). Multiple sclerosis aetiology–an Epstein-Barr virus hypothesis. *Med Hypothesis* **25:** 93–97.