REVIEW Transient virus infection and multiple sclerosis

G. J. Atkins[1]*, S. McQuaid[2], M. M. Morris-Downes[1], S. E. Galbraith[1], S. Amor[3], S. L. Cosby[2] and B. J. Sheahan[4]

[1]*Department of Microbiology, Moyne Institute of Preventive Medicine, Trinity College, Dublin 2, Ireland*
[2]*Department of Neuropathology, Institute of Pathology, Royal Victoria Hospital, Grosvenor Road, Belfast BT12 6BL, Northern Ireland*
[3]*Department of Neurodegenerative Disorders, Charing Cross Hospital, Fulham Palace Road, London W6 8RF, UK*
[4]*Department of Veterinary Pathology, Faculty of Veterinary Medicine, University College Dublin, Ballsbridge, Dublin 4, Ireland*

SUMMARY

Multiple sclerosis (MS) is a chronic, demyelinating disease of the CNS in which autoimmunity to myelin plays a role in pathogenesis. The epidemiology of MS indicates that it may be triggered by a virus infection before the age of adolescence, but attempts to associate a specific virus with MS have produced equivocal results. Many studies of the aetiology of MS have postulated that a persistent virus infection is involved, but transient virus infection may provide a plausible alternative mechanism that could explain many of the inconsistencies in MS research. The most studied animal model of MS is chronic relapsing experimental autoimmune encephalomyelitis (CREAE), which is induced in susceptible animals following injection of myelin components. While CREAE cannot provide information on the initiating factor for MS, it may mimic disease processes occurring after an initial trigger that may involve transient virus infection. The disease process may comprise separate triggering and relapse phases. The triggering phase may involve sensitisation to myelin antigens as a result of damage to oligodendrocytes or molecular mimicry. The relapse phase could be similar to CREAE, or alternatively relapses may be induced by further transient virus infections which may not involve infection of the CNS, but which may involve the recrudescence of anti-myelin autoimmunity. Although current vaccines have a high degree of biosafety, it is suggested that the measles-mumps-rubella vaccine in particular could be modified to obviate any possibility of triggering anti-myelin autoimmunity. Copyright © 2000 John Wiley & Sons, Ltd.

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INTRODUCTION

The possible involvement of viruses in the aetiology of multiple sclerosis (MS) is a subject that has created much controversy. From epidemiological studies based on immigration data and the occurrence of clusters, it has been suggested that an environmental factor(s) triggers MS before the age of adolescence, while symptoms of the disease are not observed until years later. It is also apparent that there is a genetic susceptibility to MS, so a hypothesis for the aetiology of MS formulated on this evidence is that it is a disease triggered by an environmental factor in genetically susceptible individuals during childhood [1–8].

Circumstantial evidence suggests that the environmental factor in MS could be a virus [7–14]. Several human and animal virus infections are characterised by the CNS demyelination that is also characteristic of MS (Table 1). These include subacute sclerosing panencephalitis (SSPE), caused by a persistent measles virus infection, and human T cell lymphotropic virus-I (HTLV-I)-associated myelopathy, which is a slowly progressive neurological disease characterised by

*Correspondence to: Professor G. J. Atkins, Department of Microbiology, Moyne Institute, Trinity College, Dublin 2, Ireland.
E-mail: gatkins@tcd.ie*

Abbreviations used:
CREAE, chronic relapsing experimental autoimmune encephalomyelitis; EAE, experimental autoimmune encephalomyelitis; HHV6, human herpesvirus 6; HTLV-I, human T-cell lymphotropic virus type I; MBP, myelin basic protein; MMR, measles-mumps-rubella; MOG, myelin oligodendrocyte protein; MS, multiple sclerosis; PML, progressive multifocal leukoencephalopathy; SFV, Semliki Forest virus; SSPE, subacute sclerosing panencephalitis; TMEV, Theiler's murine encephalomyelitis virus

Table 1. Some viruses associated with demyelinating disease in man and animals

Virus family	Species	Host	Type of infection	Disease induced
Paramyxovirus	Measles	Man	Transient	Encephalitis
			Persistent	Subacute sclerosing panencephalitis
		Rat	Persistent	Encephalomyelitis
	Canine distemper	Dog	Persistent	Encephalitis
Togavirus	Rubella	Man	Persistent	Progressive rubella panencephalitis
	Semliki Forest	Mouse	Transient	Encephalitis
Picornavirus	Theiler's	Mouse	Persistent	Encephalomyelitis
Coronavirus	Mouse hepatitis	Mouse	Persistnt	Encephalomyelitis
	JHM	Rat	Persistent	Encephalomyelitis
Retrovirus	HTLV-1	Man	Persistent	Myelopathy
	Visna	Sheep	Persistent	Encephalomyelitis
Papovavirus	JC	Man	Persistent	Progressive multifocal leukoencephalopathy

inflammatory infiltrates and demyelination in the CNS, and is caused by an exogenous retrovirus. Many viruses have been implicated in MS itself [13,14] (Table 2), including measles virus and more recently EBV, [15] human herpesvirus 6 (HHV6) [16] and human endogenous retroviruses [17]. There is also evidence to suggest that some of the relapses which are characteristic of most MS cases are preceded by virus infections [18–20]. For example, a recent report demonstrated a significantly higher exacerbation rate in MS patients following influenza virus infection, suggesting that influenza infection may trigger relapses [21]. Evidence for the involvement of virus infection in MS also comes from several animal diseases in which persistent virus infection gives rise to demyelination [10,11,22–24]. While these studies point to a viral aetiology of MS, no virus has yet been definitely associated with this disease despite intensive effort.

One possible explanation for the equivocal results obtained for identification of viruses

Table 2. Some viruses which have been associated with the triggering or exacerbation of multiple sclerosis

Virus family	Species	Type of infection implicated
Paramyxovirus	Measles	Persistent
	Canine distemper	Persistent
	SV5	Persistent
Togavirus	Rubella	Persistent
Retrovirus	Human T-cell lymphotropic	Persistent
	Endogenous	Persistent (as provirus)
Papovavirus	JC	Persistent
Herpesvirus	HHV6	Persistent
	EBV	Latent/superinfection
Rhabdovirus	Rabies vaccine	Antigen
Myxovirus	Influenza (including vaccine)	Transient/antigen
Hepadnavirus	Hepatitis B vaccine	Antigen
Unknown	Common uncharacterised infections	Transient

associated with MS is that, while the disease is triggered by a virus infection, the subsequent course of the disease may not require virus persistence. It is probable that virus persistence does occur in some cases, but it may or may not be relevant to the progression of the disease. It is also possible that a number of different viruses, rather than one specific type, may act as a trigger for MS in susceptible individuals.

Another important factor that requires consideration is the heterogeneity in the detailed neuropathology and clinical disease progression in MS, suggesting that the disease may not have a single aetiology. It is possible that primarily progressive MS and relapsing remitting MS are two distinct disease entities [25]. MS may in fact be divided into at least five disease subtypes on the basis of detailed analysis of neuropathology [26,27], and it is possible that only some of these may have viral involvement.

The main assumption that has been made in considering the possible viral aetiology of MS is that a persistent infection by one virus is involved. Here we review the evidence for transient virus infection, with a variety of agents, as an alternative to persistent infection in the aetiology of MS.

ANIMAL MODELS

Autoimmune models

In an attempt to understand the aetiology of MS, animal models have been used. The most studied autoimmune model of MS is experimental autoimmune encephalomyelitis (EAE), which may be induced in a number of animal species by immunisation with myelin antigens or transfer of myelin-reactive class II restricted CD4+ T lymphocytes [10]. The type of EAE induced is dependent on the immunisation protocol, animal strain and antigen used. In some cases the protocol results in an acute monophasic disease and in others a chronic relapsing disease (CREAE) occurs. CREAE resembles MS because animals develop accumulating neurological deficit following each disease phase. EAE is characterised by inflammatory infiltration of the CNS associated with lesions of demyelination, and the topography of lesions within the CNS is dependent on the nature and origin of the autoantigen [28]. The extent of demyelination varies with the protocol used but is more pronounced when myelin-

specific antibodies are injected following transfer of MBP-specific T cells or at the onset of spinal cord homogenate-induced disease [29,30]. The possible role of anti-myelin antibodies is supported by the findings of myelin reactive antibodies in the blood of MS patients, and recently autoantibodies directed to myelin basic protein (MBP) and myelin oligodendrocyte protein (MOG) peptides have been detected within lesions of demyelination in the CNS of MS patients [31]. While antibodies may be involved in the development of demyelination, many other immunological mechanisms, including the release of inflammatory mediators of disease such as TNF-α and reactive oxygen species, or the action of cytotoxic T-lymphocytes, may play a role in the myelin damage [32].

The chronicity of the disease both in CREAE and possibly MS may be due to the perpetuation of autoimmune responses by determinant spreading. This process occurs as a result of myelin damage in the initial phase of disease, when new myelin antigens are released. The release of such antigens may subsequently induce new T-cell specificities, some of which may be pathogenic [33].

Virus models

In contrast to EAE are the virus-induced models, in which both natural and experimental infections of animals result in widespread demyelination and neurological deficit. The naturally occurring diseases include canine distemper virus of dogs, and visna virus infection of sheep, both of which have been used to study the mechanism of virus-induced demyelination as models for MS. Laboratory virus infections of mice and rats have also been used as models to understand the processes of virus-induced demyelination. These experimental models include murine coronavirus, where persistent virus infection induces an EAE-like disease [22,34]. Myelin reactive T cells are also observed following experimental infection of rats with measles virus and some of these T cell lines are encephalitogenic, supporting the hypothesis that a virus trigger can lead to autoimmunity and myelin damage [35–37].

Another virus model of MS is Theiler's murine encephalomyelitis virus (TMEV) infection of mice, which produces a chronic demyelinating disease associated with virus persistence [10,11,22,23,38].

Autoimmune responses to myelin antigens are observed following infection with TMEV, and while they may not play a major role in the initiation of demyelination, autoimmunity to myelin components probably contributes to lesion progression in chronically diseased animals [23].

An alternative model of virus-induced demyelination is Semliki Forest virus (SFV) infection of mice [39,40]. SFV induces acute immune-mediated demyelination in mice, which is repaired and does not recur in most mouse strains. The initial immune-mediated demyelination induced by SFV infection may be due to targeting of SFV-infected oligodendrocytes by cytotoxic T-cells. It is clear, however, that T-cell responses to MBP [41–43] and to a range of encephalitogenic myelin epitopes (M. Morris-Downes and S. Amor, unpublished data) are also induced. A MOG peptide showing sequence similarity to the SFV E2 envelope protein, as well as the E2 peptide itself, has been shown to induce CREAE in mice, and it has been suggested that this molecular mimicry may contribute to SFV-induced demyelination [44].

In most mouse strains, for example in BALB/c mice, myelin repair is complete by 3 months after avirulent SFV infection. However, in SJL mice, small lesions of demyelination persist up to a year after infection and some of these lesions show active inflammatory demyelination. Long-term lesions do not correlate in individual mice with persistence of the virus genome, but do correlate with expression of the cytokines TNF-α or IFN-γ [45,46]. Expression of these cytokines in the CNS is undetectable in BALB/c mice by 3 months after infection, but continues in most SJL mice beyond a year after infection [45]. SJL mice, which are susceptible to EAE, appear to have a defect in innate suppression of immune responses, which is associated with secretion of lower levels of the regulatory cytokine TGF-β [41]. Prior infection with SFV predisposes mice to EAE, even in strains that are normally resistant to EAE, and infection with SFV has been shown to exacerbate EAE [42,47,48].

It is clear that the demyelination characteristic of SFV infection is immune-mediated and it appears that this may be triggered by virus infection of oligodendrocytes early in infection. Such infection may induce damage to the infected cells and the subsequent release of myelin antigens by the induction of oligodendrocyte cell death [49]. SFV-infected oligodendrocytes could also be targeted by CD8+ T-lymphocytes [50,51] or alternatively, uninfected oligodendrocytes and/or myelin in the vicinity of aggressive immune responses may be damaged by the action of cytokines such as TNF-α [43,51]. Either way, normally sequestered myelin antigens are likely to be presented to the immune system to induce autoimmunity to myelin.

A possible mechanism whereby recrudescence of immunity in the CNS could be triggered by a virus infection is suggested by a study on influenza virus infection of mice. Following an initial CNS infection, subsequent infections led to the long-term persistence of activated cytotoxic T-lymphocytes in the brain parenchyma, long after the infection was cleared [52]. Another study that does not involve virus infection, but may nonetheless provide information concerning the possible viral aetiology of MS, concerns the exacerbation of brain damage following EAE induction. If a brain cryolesion is inflicted on rats after induction of EAE by injection of myelin components, EAE lesions are enhanced [53]. This may be consistent with the proposal that the brain damage caused by virus infection could enhance EAE in a manner that is not specific to the virus.

CLINICAL STUDIES

Evidence for the involvement of specific viruses in MS comes from serological studies and direct virus detection. It was found many years ago that antibody levels to several common viruses are elevated in MS patients, but it is not clear whether this is related to the cause of MS or an epiphenomenon. Many viruses have been detected in CNS autopsy tissue from MS patients [13–17] (Table 2); many such claims have either been retracted or have not been confirmed by other workers. The inconsistent detection of viruses in CNS autopsy tissue from MS patients is illustrated by a study in which brain samples from 8 cases of MS and 56 controls were examined by *in situ* hybridisation [54]. Samples were examined for genomic RNA sequences of measles virus, canine distemper virus, rubella virus and simian virus 5, all viruses that have been implicated in MS. Positive hybridisation was

detected in two of the MS cases for measles virus only, but was also detected in one of the controls. The latter was a neurological control that was a case of disseminated CMV infection, but which showed demyelination that is uncharacteristic of CMV infection [54]. In subsequent investigation, the number of MS cases found to be positive for measles virus RNA has increased to 4 out of 14 cases examined (S. McQuaid and L. Cosby, unpublished data). In another study, measles virus RNA could not be detected by RT-PCR in peripheral blood leucocytes from MS patients [55]. Measles, mumps and rubella virus RNA has been shown to be absent from brain autopsy tissue from MS patients by RT-PCR [56]. These and other studies indicate that most cases of MS are not associated with persistent virus infection, at least with the viruses tested, but a minority of cases may be associated with the persistence of measles virus in the CNS. More recently attention has focused on HHV6 [16], EBV [57] and the MS-associated retroviruses [17]. While causal associations have yet to be definitely made with MS, it is possible that infection with a single virus alone is insufficient for the development of MS and dual infections such as retrovirus and EBV are required [15].

Some positive evidence is available, however, linking transient virus infection with MS or exacerbations of MS. Common virus infections, identified by their symptoms rather than by virus detection, have been associated with exacerbations of MS [18,20]. Apart from post-vaccination encephalitis, rabies vaccination has been associated with a disease that is indistinguishable from MS at autopsy [12], and it has been shown that the Semple vaccine strain of rabies virus can induce anti-myelin autoimmunity [58]. Recent studies have suggested that both influenza infection and influenza vaccination are associated with an increased frequency of exacerbations of MS [21]. Like the evidence for viruses in MS, the data are controversial, since other studies have failed to find an association between exacerbations of MS and influenza vaccination [59].

POSSIBLE MECHANISMS OF PATHOGENESIS BASED ON TRANSIENT VIRUS INFECTION

A summary of possible mechanisms for the involvement of transient virus infections in stimulating anti-myelin autoimmunity is shown in Figure 1. It is possible that sensitisation to myelin antigens may follow an initial trigger involving a virus infection. Subsequently, recrudescence of anti-myelin autoimmunity could occur during relapse phases induced by further transient virus infections. The anti-myelin autoimmunity may only produce a threshold of damage to myelin, resulting in clinical signs and symptoms, in a minority of patients with a genetic susceptibility to MS. This may involve an inability to suppress anti-myelin autoimmunity.

The initial phase could result in damage to myelin and the release of normally sequestered myelin antigens, which would then prime the immune system to induce anti-myelin autoimmunity. In the case of a virus infection, this initial phase could involve damage to oligodendrocytes. Such damage could be caused by direct infection of oligodendrocytes and the induction of cell
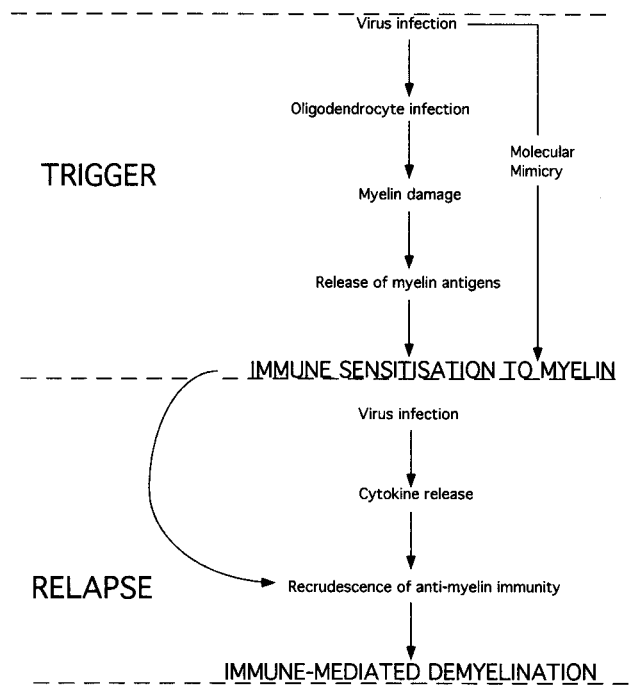

Figure 1. Diagram of postulated mechanisms of generation of anti-myelin autoimmunity, triggered and/or exacerbated by transient virus infections. Clinical signs of MS may only occur in individuals with an inherited genetic defect, possibly in the suppression of immune responses to myelin. A central feature of this hypothesis is the peripheral stimulation of anti-myelin immunity within the CNS. This peripheral stimulation could occur by cytokine secretion in response to a heterologous antigen, or by molecular mimicry

death mechanisms, by targeting of virus-infected oligodendrocytes by cytotoxic T-cells, or by other damage mediated by cytokines or antibody.

An alternative mechanism of priming of the immune system to myelin antigens may be molecular mimicry. Many bacterial and viral proteins share sequence and structural homologies with known autoantigens. Several viruses share part of the sequence of myelin proteins in their genome [60,61], and a number of peptides based on these sequences activated myelin-specific T cell clones [61]. Sequence similarity between MBP and hepatitis B virus exists, and this peptide sequence induced EAE in rabbits [62]; also, a rubella peptide shows sequence similarity to myelin proteolipid protein [63]. A mechanism similar to molecular mimicry may be the incorporation of self-antigens into the envelope of budding viruses leading to autosensitisation. If vesicular stomatitis virus, a model enveloped virus, is grown in MBP-expressing cell cultures it is highly efficient in triggering T-cell responses to MBP, both *in vitro* and *in vivo* [64]. Thus, viruses could act as presenters of host antigen to the immune system.

This initial triggering phase could be followed by a relapse phase in which reactivation of myelin-specific T-cells already present in the CNS occurs, or alternatively myelin-reactive immunity is induced outside the CNS and infiltration of the brain parenchyma by lymphocytes and other components of the immune system occurs. In either case, such activation may be a result of a transient virus infection which compromises the blood–brain barrier and leads to further influx of cells and soluble components [65,66].

A possible mechanism for the recrudescence of anti-myelin autoimmunity, as well as those mentioned above for the triggering phase, may be the activation of myelin-reactive T-cells by viral superantigens. Such activation relies on the pre-existence of myelin reactive T cells but does not occur via the classical T-cell receptor : peptide : MHC interaction. Instead it involves the polyclonal activation of T cells through the Vβ element of the T cell receptor. It has been demonstrated that EAE relapses may be induced with staphylococcal superantigens, although injection of the same superantigens into naive mice failed to induce EAE [67]. It is feasible that viruses

may also act in this way, as has been shown for murine mammary tumour virus [68].

If the proposed triggering mechanism is correct, then definitive evidence for it could not be obtained from MS autopsy or biopsy tissue, because the myelin damage resulting in the triggering event probably occurred some time previously and only the consequences of this would be evident. Also, if the damage were a result of virus infections, it may not be specific to any one virus, as at least in experimental infection many infections may give rise to a similar pathology, suggesting that any virus (including non-neurotropic viruses) that induces myelin sensitisation could act as a trigger.

With regard to the activity of known human viruses in the induction of myelin damage, there is evidence that virus infections associated with CNS demyelination can cause damage to oligodendrocytes. Measles virus has been implicated in MS [54], and virus infection of oligodendrocytes is seen in cases of SSPE [69], which is a rare human demyelinating disease caused by a persistent infection with measles virus (Figure 2A). Similarly, progressive multifocal leucoencephalopathy (PML) is a rare human demyelinating disease caused by persistent infection with a human papovavirus. This virus has also been implicated in MS [70], and virus infection of oligodendrocytes is seen in this disease (Figure 2B). In the SFV model, infection of oligodendrocytes occurs early in infection, and is followed by acute immune-mediated demyelination [71,72] (Figure 2E, F). Virus infection of oligodendrocytes by SFV is also seen in rat mixed glial cell cultures [73] (Figure 2C, D). The damage to oligodendrocytes induced by SFV infection may be due to induction of programmed cell death or apoptosis, since this is induced in cultured rat oligodendrocytes infected by SFV [74]. However, it has also been proposed that some mature oligodendrocytes in the animal survive SFV infection, and are capable of carrying out the remyelination observed later in the disease [75]. Such virus-infected oligodendrocytes could be targeted by CD8 + T-lymphocytes [49], which would also result in myelin damage. Rubella virus, a togavirus like SFV, and another virus that has been implicated in MS [76], shows tropism for oligodendrocytes in rat mixed glial cell cultures [77]. In canine distemper, which is a
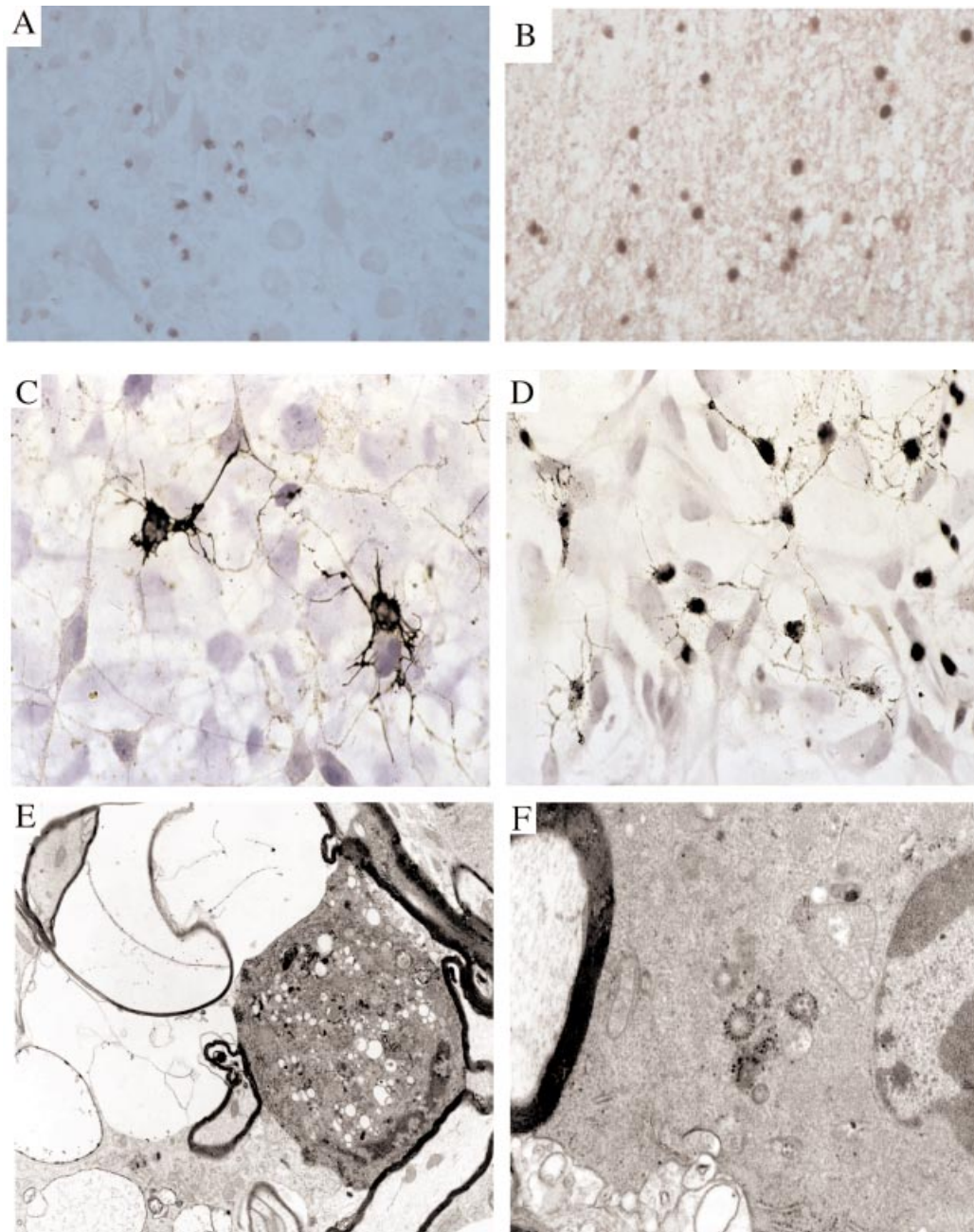
Figure 2. Infection of oligodendrocytes by viruses which have been implicated in MS (JC virus and measles) or which trigger immune-mediated demyelination in an animal model (Semliki Forest virus). (A, B) Detection of virus in cells with the morphological characteristics of oligodendrocytes in the white matter from human CNS tissues by *in situ* hybridisation. (A) Measles virus RNA in subacute sclerosing panencephalitis (original magnification ×400). (B) JC virus DNA in progressive multifocal leucoencephalopathy (×400). (C, D) Antibody staining (black/brown) of rat mixed glial cell cultures using the immuno-gold silver staining technique. (C) Uninfected cells stained with anti-galactocerebroside, an oligodendrocyte marker, showing positive staining of oligodendrocytes above a layer of astrocytes. (D) Mixed glial cell culture 24 h after infection with the M9 mutant of Semliki Forest virus, stained with anti-SFV antibody; the upper oligodendrocyte layer only is immunostained. Counterstained with haematoxylin (×400). (E) Swollen oligodendrocyte and intra-myelinic vacuolation in the mid-brain of a BALB/c mouse 5 days after intraperitoneal infection with the M9 mutant of Semliki Forest virus. The nucleus of the oligodendrocyte is marginated and there is widespread cytoplasmic vacuolation. Electron micrograph (×4500). (F) Virus particles and nucleocapsids in the cytoplasm of an oligodendrocyte. BALB/c mouse, mid-brain, 5 days after intraperitoneal infection with the M9 mutant of Semliki Forest virus. Electron micrograph (×20 000)

disease of dogs caused by a virus that has also been implicated in MS in the past [13], damage to oligodendrocytes also occurs, although this does not appear to be associated with productive virus infection, which mainly occurs in astrocytes [78,79]. Lymphoproliferative responses to MBP have been detected in patients with encephalitis after measles virus, VZV or rubella virus infection, and after rabies vaccination [80]. In the case of measles virus, a T cell-mediated response to MBP was induced following intracerebral infection of rats; such sensitised T-cells showed no cross-reactivity to measles virus, but could induce EAE by adoptive transfer to naïve recipients. Also, a normally non-encephalitogenic MBP peptide induced EAE in measles virus-infected rats [37]. This is further evidence that virus infection of the CNS can sensitise to subsequent autoimmune demyelination.

The chronic immune-mediated demyelination that occurs in MS could be maintained by either of two mechanisms following sensitisation of the immune system to myelin antigens after the initial trigger. One mechanism could be similar to CREAE. Another possibility is that the relapses characteristic of most cases of MS could be induced by further transient virus infections, which may not necessarily involve infection of the CNS.

The clinical evidence that transient virus infections could be implicated in relapses of MS is mentioned above; there is also indirect evidence that such a mechanism could operate. Many virus infections lead to the production of pro-inflammatory cytokines such TNF-α or IFN-γ. Infection of SJL mice with SFV results in the expression of these cytokines in the CNS for more than a year after the initial infection [45]. It is known that administration of IFN-γ to MS patients results in exacerbation of the disease [81]. Also, the demyelination in measles virus encephalitis cannot be associated with the presence of measles virus antigen in the CNS at time of death, although it is not clear whether virus infection of the CNS occurred earlier in the course of the disease [82]. It is possible that administration of IFN-β, which is a treatment currently used for MS with partial success [83], could ameliorate the effects of some transient infections that may trigger exacerbations of MS. However, adminis-tration of IFN-β may also have an immunomodu-latory effect [84].

It is possible that virus infection could induce secretion of pro-inflammatory cytokines that could penetrate the CNS parenchyma from the blood and lead to the recrudescence of anti-myelin autoimmunity by reactivation of previously primed T-cells. It is also possible that such myelin-reactive T-cells could penetrate the brain parenchyma following damage to the blood–brain barrier caused by transient virus infection [66], or following the secretion of pro-inflammatory cyto-kines and/or chemokines by cells within the CNS (astrocytes and microglia as well as lymphocytes) [85]. If such a mechanism were to operate, it may not be virus-specific, but may be induced by many different transient infections.

CONCLUSIONS

Much of the evidence cited to support the idea that MS is associated with a specific persistent virus infection of the CNS has been challenged, or shown to be incorrect. It is possible that there are still unknown viruses which infect the CNS and which may contribute to MS pathogenesis. Current candidates for persistent virus infection associated with the aetiology of MS include HHV6, EBV and human endogenous retroviruses. Whether these viruses are indeed involved in the pathogenesis of at least some cases of MS is still unknown. However, MS may comprise a group of diseases of different aetiologies: persistent virus infection may represent one such aetiology, and the mechanisms triggered by transient infection described here may represent another. Since the circumstantial evidence that MS is associated with a virus infection has not been refuted, it seems reasonable to consider the possibility that most cases of MS are not associated with a persistent virus infection, but that other mechanisms invol-ving transient virus infection could operate.

If MS is induced and/or exacerbated by transient virus infection, there are several corol-laries that may explain the equivocal results obtained for the association of viruses with MS. Firstly, it would not be possible in most cases to detect virus in autopsy tissue from MS patients, or in biopsy samples taken after the initial triggering phase. Secondly, virus infections need not be specific, and it is possible that a range of viruses with common properties could be involved in

either the triggering or maintenance phases. Thirdly, it is probable that, if viruses are involved in triggering and/or maintaining MS, that these are common viruses that only have this effect in a minority of genetically susceptible individuals.

If transient virus infection is involved in the triggering or maintenance of the antimyelin autoimmunity that is a risk factor for MS, the role of vaccination must be considered. Of the common diseases for which vaccines are available, measles, mumps and rubella viruses have all been implicated in MS [9,13,14]. There is conflicting evidence as to whether influenza or hepatitis B vaccination can trigger CNS inflammation [21,59,86,87]. However, possible mechanisms of induction of CNS inflammation may be different for hepatitis B and influenza vaccines, which are non-replicative, compared with the measles-mumps-rubella (MMR) vaccine, which is a cocktail of three replicating attenuated viruses. Wild-type measles, mumps and rubella viruses are all known to infect the CNS, and the Urabe Am 9 mumps vaccine strain, now no longer used as a vaccine component, caused CNS symptoms (aseptic meningitis) in a small minority of recipients [88–90], showing that it is neurotropic.

In developed countries, the MMR vaccine is routinely given to children to prevent disease caused by measles, mumps and rubella virus infections [89]. Although it is clear that the incidence of MS has not been significantly affected by the introduction of the vaccine, this could be explained if either the wild-type infections or the vaccine could trigger one type of MS in a minority of individuals. The mechanism by which vaccines may induce CNS inflammation could be but one of a number of different mechanisms operating as an MS trigger. The hypothesis that vaccination could trigger MS is consistent with current knowledge of the epidemiology of MS, in particular the hypothesis deduced from studies of the epidemiology of MS, that it is a disease triggered by an environmental factor exerting its effect in a minority of genetically susceptible individuals before the age of adolescence. In the UK, rubella vaccination was introduced in 1970 for girls only but was extended to both sexes in 1988 when the MMR vaccine was introduced [89]. Recently, peripheral neuropathy has been associated with rubella vaccination in which anti-MBP reactivity was prominent [91]. However, as has been

suggested for influenza [21], vaccination may be less likely to trigger MS or MS exacerbations than the virulent infection.

Vaccination has been the most successful method of controlling virus diseases [92], and current evidence indicates that the benefits of vaccination outweigh any disadvantages, for which there is no conclusive evidence at present. Thus there is no justification for the discontinuation of vaccination either in the general population or for MS patients. However, although current vaccines have a high degree of biosafety, a small risk associated with vaccination, which is much less than that associated with the wild-type infection, cannot be excluded. The evidence available at present does not indicate that vaccination can trigger MS and/or exacerbations of MS, but this possibility warrants further investigation of vaccines, particularly the MMR vaccine. In particular, the ability of vaccine strains to stimulate anti-myelin autoimmunity in a manner similar to wild-type virus strains, and as a combination vaccine, should be investigated.

It is possible that the more rational design of vaccines, based on recombinant DNA technology, could improve them and circumvent any possible problems which may be associated with current vaccines. It may be possible to replace live virus vaccines with engineered virus vaccines expressing only desired protective epitopes. The use of new genetically manipulated vaccines will create its own controversy, and the use of such vaccines can only be justified if they are demonstrably more effective and have greater biosafety than conventional vaccines currently in use. Of particular concern is a report that the plasmid backbone used to construct naked DNA vaccines, which have been suggested as replacements for current vaccines [93], has been shown to potentiate EAE. The mechanism appears to be the induction of Th1-promoting cytokines [94]. However, DNA vaccines have poor immunogenicity and persist for long periods in the host tissue [95] (M. M. Morris-Downes and G. J. Atkins, unpublished results). Other types of prototype recombinant vaccines include naked RNA vaccines and recombinant suicide particles based on the SFV genome [40,96]. Such vaccines stimulate immune responses more efficiently than naked DNA vaccines [97], and induce apoptosis which may lead to the removal of the vaccine from inoculated

tissue [98]. This may lead to a more transient stimulation of pro-inflammatory cytokine synthesis than is possible either with conventional attenuated vaccines or naked DNA vaccines. If the mechanism of induction of myelin damage by viruses were known, it may be possible to express only protective epitopes using such vaccine vectors. It may also be possible to omit known encephalitogenic sequences from such vaccines, and to circumvent any virus functions, such as a tropism for oligodendrocytes, which lead to myelin damage.

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REFERENCES

1. Alter M, Cendrowski W. Multiple sclerosis and childhood infections. *Neurology* 1976; **26**: 201–204.
2. Dean G. Serendipity and multiple sclerosis epidemiology. In *Multiple Sclerosis in Europe. An Epidemiological Update*, Firnhaber W, Lauer K (eds). Leuchtturm-Verlag LTVPress: Darmstadt, 1994; 28–40.
3. Gaudet JPC, Hashimoto L, Sadovnik AD, Ebers GC. Is sporadic MS caused by an infection of adolescence and early childhood? A case–control study of birth order position. *Acta Neurol Scand* 1995; **91**: 19–21.
4. James WH. Further evidence in support of the hypothesis that one cause of multiple sclerosis is childhood infections. *Neuroepidemiology* 1988; **7**: 130–133.
5. Kurland LT. The evolution of multiple sclerosis epidemiology. *Annal Neurol* 1994; **36**: S2–5.
6. Kurtzke JF. Epidemiologic evidence for multiple sclerosis as an infection. *Clin Microbiol Rev* 1993; **6**: 382–427.
7. Millar JHD. *Multiple Sclerosis, a Disease Acquired in Childhood*. CC Thomas: Springfield, Illinois, 1971.
8. Sadovnik AD, Ebers GC. Genetic factors in the pathogenesis of MS. *Int MS J* 1994; **1**: 16–24.
9. Allen IV, Brankin B. Pathogenesis of multiple sclerosis—the immune diathesis and the role of viruses. *J Neuropathol Exp Neurol* 1993; **52**: 95–105.
10. Fazakerley JK, Amor S, Nash AA. Animal model systems of MS. In *Molecular Biology of Multiple Sclerosis*, Russell WC (ed.). John Wiley & Sons Ltd: London, 1997; 255–273.
11. Fazakerley JK, Buchmeier MJ. Pathogenesis of virus induced demyelination. *Adv Virus Res* 1993; **42**: 249–324.
12. Poser CM. Pathogenesis of multiple sclerosis. A critical reappraisal. *Acta Neuropathol* 1986; **71**: 1–10.
13. Russell WC. Viruses and MS. In *Molecular Biology of Multiple Sclerosis*. Russell WC (ed.). John Wiley & Sons Ltd: London, 1997; 243–254.
14. Waksman BH, Reingold SC. Viral etiology of multiple sclerosis: where does the truth lie? *TINS* 1986; **9**: 388–391.
15. Haahr S, Sommerlund M, Christensen T, Jensen AW, Hansen HJ, Moller-Larsen A. A putative new retrovirus associated with multiple sclerosis and the possible involvement of Epstein–Barr virus in this disease. *Ann N Y Acad Sci* 1994; **724**: 148–156.
16. Moore P. Herpesvirus 6 might trigger multiple sclerosis. *Lancet* 1997; **350**: 1685–1687.
17. Christensen T, Sorensen PD, Riemann H, Hansen HJ, Moller Larsen A. Expression of sequence variants of endogenous retrovirus RGH in particle form in multiple sclerosis. *Lancet* 1998; **352**: 1033–1035.
18. Andersen O. Viral infections trigger multiple sclerosis relapses—a prospective seroepidemiological study. *J Neurol* 1993; **240**: 417–422.
19. Lycke J, Svennerholm B, Hjelmquist E, *et al.* Acyclovir treatment of relapsing-remitting multiple sclerosis. A randomized, placebo-controlled, double-blind study. *J Neurol* 1996; **243**: 214–224.
20. Sibley WA, Bamford CR, Clark K. Clinical viral infections and multiple sclerosis. *Lancet* 1985; **1**: 1313–1315.
21. De Keyser J, Zwanikken C, Boon M. Effects of influenza vaccination and influenza illness on exacerbations in multiple sclerosis. *J Neurol Sci* 1998; **159**: 1–3.
22. Atkins GJ, Balluz IM, Glasgow GM, *et al.* Analysis of the molecular basis of neuropathogenesis of RNA viruses in experimental animals: relevance for human disease? *Neuropath Appl Neurobiol* 1993; **20**: 91–102.

23. Miller SD, Vanderlugt CL, Begolka WS, *et al*. Persistent infection with Theiler's virus leads to CNS autoimmunity via epitope spreading. *Nature Med* 1997; **3**: 1133–1136.

24. Watanabe R, Wege H, ter Meulen V. Adoptive transfer of EAE-like lesions from rats with coronavirus-induced demyelinating encephalomyelitis. *Nature* 1983; **305**: 150–153.

25. Ollerup O, Hiller J, Fredrikson S, *et al*. Primarily chronic progressive and relapsing/remitting multiple sclerosis: two immunologically distinct entities. *Proc Natl Acad Sci USA* 1989; **86**: 7113–1777.

26. Lassman H. Neuropathology in multiple sclerosis: new concepts. *Multiple Sclerosis* 1998; **4**: 93–98.

27. Lucchinetti CF, Brück W, Rodriguez M, Lassman H. Distinct patterns of multiple sclerosis pathology indicates heterogeneity in pathogenesis. *Brain Pathol* 1996; **6**: 259–274.

28. Berger T, Weerth S, Kojima K, Linington C, Wekerle H, Lassmann H. Experimental autoimmune encephalomyelitis: The antigen specificity of T lymphocytes determines the topography of lesions in the central and peripheral nervous system. *Lab Invest* 1997; **76**: 355–364.

29. Linington C, Bradl M, Lassmann H, Brunner C, Vass K. Augmentation of demyelination in rat acute allergic encephalomyelitis by circulating mouse monoclonal antibodies directed against a myelin/oligodendrocyte glycoprotein. *Am J Pathol* 1988; **130**: 443–454.

30. Morris MM, Piddlesden S, Groome N, Amor S. Anti-myelin antibodies modulate experimental allergic encephalomyelitis in Biozzi ABH mice. *Biochem Soc Trans* 1997; **25**: 168S.

31. Genain CP, Cannella B, Hauser SL, Raine CS. Identification of auto-antibodies associated with myelin damage in multiple sclerosis. *Nature Med* 1999; **5**: 170–175.

32. Steinman L. Multiple sclerosis: a coordinated immunological attack against myelin in the central nervous system. *Cell* 1996; **85**: 299–302.

33. McRae BL, Vanderlugt CL, Dal Canto MC, Miller SD. Functional evidence for epitope spreading in the relapsing pathology of experimental autoimmune encephalomyelitis. *J Exp Med* 1995; **182**: 75–85.

34. Watanabe R, Wege H, ter Meulen V. Adoptive transfer of EAE-like lesions from rats with coronavirus-induced demyelinating encephalomyelitis. *Nature* 1983; **305**: 150–153.

35. Liebert UG, Hashim GA, ter Meulen V. Characterization of measles virus-induced autoimmune reactions against myelin basic protein in Lewis rats. *J Neuroimmunol* 1990; **29**: 139–147.

36. Liebert UG, Linington C, ter Meulen V. Induction of autoimmune reactions to myelin basic protein in measles virus encephalitis in Lewis rats. *J Neuroimmunol* 1988; **17**: 103–118.

37. Liebert UG, ter Meulen V. Synergistic interaction between measles virus infection and myelin basic protein peptide-specific T cells in the induction of experimental allergic encephalomyelitis in Lewis rats. *J Neuroimmunol* 1993; **46**: 217–223.

38. Rodriguez M, Pavelko KD, Njenga MK, Logan WC, Wettstein PJ. The balance between persistent virus infection and immune cells determines demyelination. *J Immunol* 1996; **157**: 5699–5709.

39. Atkins GJ, Sheahan BJ, Dimmock NJ. Semliki Forest virus infection of mice: a model for the genetic and molecular analysis of viral pathogenicity. *J Gen Virol* 1985; **66**: 395–408.

40. Atkins GJ, Sheahan BJ, Liljeström P. The molecular pathogenesis of Semliki Forest virus: a model virus made useful?. *J Gen Virol* 1999; **80**: 2287–2297.

41. Mokhtarian F, Shi Y, Zhu PF, Grob D. Immune responses, and autoimmune outcome, during virus infection of the central nervous system. *Cellular Immunol* 1994; **157**: 195–210.

42. Mokhtarian F, Swoveland P. Predisposition to EAE induction in resistant mice by prior infection with Semliki Forest virus. *J Immunol* 1987; **138**: 3264–3268.

43. Mokhtarian F, Wesselingh S, Choi S, *et al*. Production and role of cytokines in the CNS of mice with acute viral encephalomyelitis. *J Neuroimmunol* 1996; **66**: 11–22.

44. Mokhtarian F, Zhang Z, Shi Y, Gonzales E, Sobel RA. Molecular mimicry between a viral peptide and a myelin oligodendrocyte glycoprotein peptide induces autoimmune demyelinating disease in mice. *J Neuroimmunol* 1999; **95**: 43–54.

45. Donnelly SM, Sheahan BJ, Atkins GJ. Long-term effects of Semliki Forest virus infection in the mouse central nervous system. *Neuropath Appl Neurobiol* 1997; **23**: 235–241.

46. Smyth JMB, Sheahan BJ, Atkins GJ. Multiplication of virulent and demyelinating Semliki Forest virus in the mouse central nervous system—consequences in BALB/c and SJL mice. *J Gen Virol* 1990; **71**: 2575–2583.

47. Wu L-X, Makela MJ, Roytta M, Salmi A. Effect of viral infection on experimental allergic encephalomyelitis in mice. *J Neuroimmunol* 1988; **18**: 139–153.

48. Erälinna JP, Soiluhänninen M, Roytta M, *et al*. Facilitation of experimental allergic encephalomyelitis by irradiation and virus infection: role of inflammatory cells. *J Neuroimmunol* 1994; **55**: 81–90.

49. Subak-Sharpe I, Dyson H, Fazakerley JK. *In vivo* depletion of CD8+ T cells prevents lesions of demyelination in Semliki Forest virus infection. *J Virol* 1993; **67**: 7629–7633.

50. Amor S, Scallan M, Morris MM, Dyson H, Fazakerley JK. Role of immune responses in protection and pathogenesis during SFV infection. *J Gen Virol* 1996; **77**: 281–291.

51. Morris MM, Dyson H, Baker D, Harbige LS, Fazakerley JK, Amor SA. Characterization of the cellular and cytokine response in the central nervous system following Semliki Forest virus infection. *J Neuroimmunol* 1997; **74**: 185–197.

52. Hawke S, Stevenson PG, Freeman S, Bangham CRM. Long-term persistence of activated cytotoxic T lymphocytes after viral infection of the central nervous system. *J Exp Med* 1998; **187**: 1575–1582.

53. Phillips MJ, Weller RO, Kida S, Iannotti F. Focal brain damage enhances experimental allergic encephalomyelitis in brain and spinal cord. *Neuropath Appl Neurobiol* 1995; **21**: 189–200.

54. Cosby SL, McQuaid S, Taylor MJ, *et al*. Examination of eight cases of multiple sclerosis and 56 neurological and non-neurological controls for genome sequences of measles virus, canine distemper virus, simian virus 5 and rubella virus. *J Gen Virol* 1989; **70**: 2027–2036.

55. Brankin B, Osman M, Herlihy L, Hawkins SA, Cosby SL. Failure to detect measles virus RNA by reverse transcription-polymerase chain reaction, in peripheral blood leucocytes of patients with multiple sclerosis. *Multiple Sclerosis* 1996; **1**: 204–206.

56. Godec MS. Absence of measles, mumps and rubella viral gene sequences from multiple sclerosis brain tissue by polymerase chain reaction. *Ann Neurol* 1992; **32**: 410–414.

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

58. Piyasirisilp S, Hemachudha T, Griffin DE. B-cell responses to myelin basic protein and its epitopes in autoimmune encephalomyelitis induced by Semple rabies vaccine. *J Neuroimmunol* 1999; **98**: 96–104.

59. Miller AE, *et al*. A multicenter, randomized, double-blind placebo-controlled trial of influenza immunization in multiple sclerosis. *Neurology* 1997; **48**: 312–314.

60. Jahnke U, Fischer EH, Alvord EC Jr. Sequence homology between certain viral proteins and proteins related to encephalomyelitis and neuritis. *Science* 1985; **229**: 282–284.

61. Wucherpfennig KW, Strominger JL. Molecular mimicry in T-cell mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. *Cell* 1995; **80**: 695–705.

62. Fujinami RS, Oldstone MB. Amino acid homology between the encephalitogenic site of myelin basic protein and virus: mechanism for autoimmunity. *Science* 1985; **223**: 1043–1045.

63. Atkins GJ, Daly EA, Sheahan BJ, Higgins DG, Sharp PM. Multiple sclerosis and molecular mimicry. *Neuropath Appl Neurobiol* 1990; **16**: 179–180.

64. Rott O, Herzog S, Cash E. Autoimmunity caused by host cell protein-containing viruses. *Med Microbiol Immunol* 1994; **183**: 195–204.

65. Kermode AG. Breakdown of the blood–brain barrier precedes symptoms and other MRI signs of new lesions in multiple sclerosis. *Brain* 1990; **113**: 1477–1489.

66. Kirk J, Zhou A-L. Viral infection at the blood–brain barrier in multiple sclerosis:—an ultrastructural study of tissues from a UK regional brain bank. *Multiple Sclerosis* 1996; **1**: 242–252.

67. Brocke S, Gaur A, Piercy C, *et al*. Induction of relapsing paralysis in experimental autoimmune encephalomyelitis by bacterial superantigen. *Nature* 1993; **365**: 642–644.

68. Held W, Waanders GA, Acha-Orbea H, MacDonald HR. Reverse transcriptase-dependent and -independent phases of infection with mouse mammary tumor virus: implications for superantigen function. *J Exp Med* 1994; **180**: 2347–2351.

69. Allen IV, McQuaid S, McMahon J, Kirk J, McConnell R. The significance of measles virus antigen and genome distribution in the CNS in SSPE for mechanisms of viral spread and demyelination. *Neuropathol Exp Neurol* 1996; **55**: 471–480.

70. Stoner GL. Polyomavirus models of brain infection and the pathogenesis of multiple sclerosis. *Brain Pathol* 1993; **3**: 213–227.

71. Atkins GJ, Sheahan BJ. Semliki Forest virus neurovirulence mutants have altered cytopathogenicity for central nervous system cells. *Infect Immun* 1982; **36**: 333–341.

72. Sheahan BJ, Gates MC, Caffrey JF, Atkins GJ. Oligodendrocyte infection and demyelination produced in mice by the M9 mutant of Semliki Forest virus. *Acta Neuropath* 1983; **60**: 257–265.

73. Atkins GJ, Sheahan BJ, Mooney DA. Pathogenicity of Semliki Forest virus for the rat central nervous system and primary rat neural cell cultures—possible implications for the pathogenesis of multiple sclerosis. *Neuropathol Appl Neurobiol* 1990; **16**: 57–68.

74. Glasgow GM, McGee MM, Mooney DA, Sheahan BJ, Atkins GJ. Death mechanisms in cultured cells infected by Semliki Forest virus. *J Gen Virol* 1997; **78**: 1559–1563.

75. Butt AM, Tutton MG, Kirvell SL, Amor S, Jenkins HG. Morphology of oligodendrocytes during demyelination in optic nerves of mice infected

with Semliki Forest virus. *Neuropath Appl Neurobiol* 1996; **22**: 540–547.

76. Nath A, Wolinsky JS. Antibody response to rubella virus structural proteins in multiple sclerosis. *Annal Neurol* 1990; **27**: 533–536.

77. Atkins GJ, Mooney DA, Fahy DA, Ng SH, Sheahan BJ. Multiplication of rubella and measles viruses in primary rat neural cell cultures: relevance to a postulated triggering mechanism for multiple sclerosis. *Neuropath Appl Neurobiol* 1991; **17**: 299–308.

78. Schobesberger M, Zurbriggen A, Summerfield A, Vandevelde M, Griot C. Oligodendroglial degeneration in distemper: apoptosis or necrosis? *Acta Neuropath* 1999; **97**: 279–287.

79. Summers BA, Appel MJG. Aspects of canine distemper and measles virus encephalomyelitis. *Neuropath Appl Neurobiol* 1994; **20**: 525–534.

80. Johnson RT, Griffin DE, Hirsch RL, *et al*. Measles encephalomyelitis—clinical and immunologic studies. *N Engl J Med* 1984; **310**: 137–141.

81. Armstrong MA. Multiple sclerosis and gamma interferon. *Lancet* 1987; **I**: 1369–1370.

82. Gendelman HE, Wolinski JS, Johnson RT, Pressman NJ, Pezeshkpour GH, Boisset GF. Measles encephalomyelitis: lack of evidence of viral invasion of the central nervous system and quantitative study of the nature of demyelination. *Ann Neurol* 1983; **15**: 353–360.

83. Paty DW. Interferon beta-1b is effective in relapsing remitting multiple sclerosis. *Neurology* 1993; **43**: 655–667.

84. Yong VW, Chabot S, Stuve O, Williams G. Interferon beta in the treatment of multiple sclerosis. Mechanism of action. *Neurology* 1997; **51**: 682–689.

85. Glabinski AR, Ranshoff RM. Chemokines and chemokine receptors in CNS pathology. *J Neurovirol* 1999; **5**: 3–12.

86. Tourbah A, Gout O, Liblau R, *et al*. Encephalitis after hepatitis B vaccination—Recurrent disseminated encephalitis or MS? *Neurology* 1999; **53**: 396–401.

87. Zipp F, Weil JG, Einhäupl KM. No increase in demyelinating diseases after hepatitis B vaccination. *Nature Med* 1999; **5**: 964–965.

88. Balraj V, Miller E. Complications of mumps vaccines. *Rev Med Virol* 1995; **5**: 219–227.

89. Salisbury DM, Begg NT. *Immunisation Against Infectious Disease*. HMSO: London, 1996.

90. Wandel G. Mumps component in combined measles, mumps and rubella vaccines. *Vaccine* 1999; **17**: 511.

91. Cusi MG, Bianchi S, Santini L, *et al*. Peripheral neuropathy associated with anti-myelin basic protein antibodies in a woman vaccinated with rubella virus vaccine. *J Neurovirol* 1999; **5**: 209–214.

92. Griffiths PD. Defending vaccines from the enemy within. *Rev Med Virol* 1999; **9**: 145–146.

93. Hassett DE, Whitton JL. DNA immunization. *Trends Microbiol* 1996; **4**: 307–312.

94. Tsunoda I, Tolley ND, Theil DJ, Whitton JL, Kobayashi H, Fujinami RS. Exacerbation of viral and autoimmune animal models for multiple sclerosis by bacterial DNA. *Brain Pathol* 1999; **9**: 481–493.

95. Polo JM, Dubenski TW. DNA vaccines with a kick. *Nature Biotechnol* 1998; **16**: 517–518.

96. Berglund P, Fleeton M, Smerdou C, *et al*. Vaccination with recombinant suicidal DNA/RNA. In *New Developments and New Applications in Animal Cell Technology*, Merten O, Perrin P, Griffiths B (eds). Kluwer Academic Publishers: Dordrecht, 1998; 583–590.

97. Fleeton MN, Liljeström P, Sheahan BJ, Atkins GJ. Recombinant Semliki Forest virus particles expressing louping ill virus antigens induce a better protective response than plasmid-based DNA vaccines or an inactivated whole particle vaccine. *J Gen Virol,* 2000; in press.

98. Glasgow GM, McGee MM, Tarbatt CJ, Mooney DA, Sheahan BJ, Atkins GJ. The Semliki Forest virus vector induces p53-independent apoptosis. *J Gen Virol* 1998; **79**: 2405–2410.