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John K Fazakerley and Robert Walker

Centre for Infectious Diseases, University of Edinburgh, Summerhall, Edinburgh, United Kingdom

A number of viruses can initiate central nervous system (CNS) diseases that include demyelination as a major feature of neuropathology. In humans, the most prominent demyelinating diseases are progressive multifocal leukoencephalopathy, caused by JC papovirus destruction of oligodendrocytes, and subacute sclerosing panencephalitis, an invariably fatal childhood disease caused by persistent measles virus. The most common neurological disease of young adults in the developed world, multiple sclerosis, is also characterized by lesions of inflammatory demyelination; however, the etiology of this disease remains an enigma. A viral etiology is possible, because most demyelinating diseases of known etiology in both man and animals are viral. Understanding of the pathogenesis of virus-induced demyelination derives for the most part from the study of animal models. Studies with neurotropic strains of mouse hepatitis virus, Theiler's virus, and Semliki Forest virus have been at the forefront of this research. These models demonstrate how viruses enter the brain, spread, persist, and interact with immune responses. Common features are an ability to infect and persist in glial cells, generation of predominantly $CD8^+$ responses, which control and clear the early phase of virus replication but which fail to eradicate the infection, and lesions of inflammatory demyelination. In most cases demyelination is to a limited extent the result of direct virus destruction of oligodendrocytes, but for the most part is the consequence of immune and inflammatory responses. These models illustrate the roles of age and genetic susceptibility and establish the concept that persistent CNS infection can lead to the generation of CNS autoimmune responses. *Journal of NeuroVirology* (2003) **9,** 148–164.

Keywords: CNS; demyelination; multiple sclerosis; virus

Introduction

There are clear examples of demyelinating diseases in humans and animals that are not associated with virus infections, for example adrenoleukodystrophy or demyelination associated with vitamin deficiency or toxins. However, many naturally occurring central nervous system (CNS) demyelinating diseases of known etiology, in man and animals, are viral in origin. Of the human demyelinating diseases, the most prominent is multiple sclerosis (MS). This is the most common neurological disease of young adults in the developed world; however, the etiology remains to be determined. For some demyelinating diseases, viruses are clearly the etiologic agent and infect the CNS, JC papovavirus (JCV) leading progressive multifocal leukoencephalopathy (PML) is an example. In other demyelinating diseases, for example postinfectious encephalomyelitis, also known as postvaccinal or perivenous encephalomyelitis, demyelination is preceded by a systemic virus infection but there is no, or only rarely, evidence of direct virus infection of the CNS. This has been observed with rabies, measles, mumps, rubella, influenza, vaccinia, and smallpox virus infections and vaccinations (Johnson, 1998). This demyelination is generally considered to be autoimmune.

The relative inaccessibility and sensitivity of the CNS precludes studies on disease pathogenesis in humans and much of the knowledge on infections and immune responses in this system has been derived from experimental animal models. For studies on mechanisms of viral demyelination, prominent

Address correspondence to Dr. John K. Fazakerley, Centre for Infectious Disease, University of Edinburgh, Summerhall, Edinburgh EH9 1QP, United Kingdom. E-mail: John.Fazakerley@ed.ac.uk

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Table 1 Demyelinating viruses

Virus	*Family*	*Host*
Semliki Forest Virus	Togaviridae	Mouse
Ross River virus		Mouse
Venezuelan equine encephalitis virus		Mouse
Mouse hepatitis virus	Coronaviridae	Mouse, rat, non-human primates
Herpes simplex I virus	Herpesviridae	Mouse, man
Measles	Paramyxovirus	Rodents
Canine distemper virus		Dog
JC virus	Papovaviridae	Man
Theiler's virus	Picornaviridae	Mouse
Maedi-visna virus	Retroviridae	Sheep
HTLV-I		Man
HIV		Man
Vesicular stomatitis virus	Rhabdoviridae	Mouse
Chandipura virus		Mouse

experimental systems include Theiler's virus, mouse hepatitis virus, and Semliki Forest virus infections of laboratory rodents. Also valuable have been experimental studies of natural infections of animals that induce demyelination, these include maedi-visna virus in sheep and canine distemper virus in dogs. Studies on human brain samples from cases of PML, subacute sclerosing panencephalitis (SSPE) and human T-cell lymphotropic virus type I (HTLV-I)–associated myelopathy (HAM), caused by JCV, measles virus, and HTLV-I, respectively, have also proved of great value in understanding events and mechanisms in CNS demyelination, though the picture of neuropathology they present is most frequently that of end-stage disease. Table 1 provides a list of viruses associated with demyelinating diseases.

It should be realized that many viruses infect the nervous system without producing demyelination. These include viruses that produce acute or subacute encephalitis, for example rabies virus and viruses that produce widespread and even life-long persistent infections, for example lymphocytic choriomeningitis virus. Tropism of infection is probably relevant here. There is considerable variation in the cell types infected by different neurotropic viruses, some are highly specific in the cell types infected, others infect several cell types. Rabies predominantly infects neurons, HIV microglial cells, and JC virus oligodendrocytes, but Theiler's virus and Herpes simplex virus can infect neurons, oligodendrocytes, astrocytes, and microglial cells. Many neurotropic viruses not associated with demyelinating diseases produce minimal infection in oligodendrocytes.

In both human and animals, most natural cases of demyelinating disease associated with virus infection are complications that occur only rarely, relative to the extent of infection. JC virus infects and persists in most adults but PML is a rare disease. Measles virus infection generally results in a systemic infection characterized by skin rash but in rare cases results in neurologic disease, measles inclusion body encephalitis (MIBE) in young adults, or SSPE in children. Visna, a demyelinating disease of sheep, is far less common than the pulmonary disease maedi caused by the same virus, maedi-visna virus. Canine distemper is rare relative to the extent of infection of the dog population. Postinfectious encephalomyelitis is a rare disease relative to the extent of the infection and vaccination. Experimental studies with the related Theiler's virus, which is also generally an enteric infection but which does cause CNS demyelinating disease in mice, indicate that only 1 in every 2000 naturally infected mice develops neurologic disease.

One explanation for the low incidence of demyelinating disease following virus infections would be a low efficiency of neuroinvasion. Certainly, viruses differ in their ability to enter the CNS. Rabies virus, one of the most deadly of viruses, always gains entry to the CNS after a peripheral infection. This is well-documented to be by travel along peripheral nerves (Murphy, 1977). The other main route of entry to the CNS is via the blood and here too some viruses can be highly efficient, for example the alphaviruses Semliki Forest virus, Sindbis virus, and Venezuelan equine encephalitis. Although it is tempting to extrapolate and assume that incidence of clinical disease is a measure of the frequency of neuroinvasion, this seems unlikely to be the case. Determining the extent of correlation between CNS infection and clinical disease is difficult. Assay of virus load in the brain following experimental extraneural inoculation of rodents indicates that it is possible for viruses to be highly efficient at neuroinvasion but to produce apparent clinical disease only rarely. The incidence of SSPE in nonimmunized populations has been estimated to be approximately one case per million children per year (Detels *et al*, 1973); however, it is possible that measles virus is more efficiently neuroinvasive than this suggests. In a study of 59 patients with acute uncomplicated measles, without any neurologic signs, 30% had a pleocytosis shortly after onset of rash, 15% had viral-specific antibodies in their cerebrospinal fluid, 10% had evidence of blood-brain barrier disturbance, and 50% had abnormal electroencephalograms (Hanninen *et al*, 1980). Neuroinvasion, at least by some viruses, may therefore be more frequent than neurologic clinical disease would suggest.

Progressive multifocal leukoencephalopathy

PML is a fatal CNS demyelinating disease of humans, most frequently associated with immunosuppression and caused by infection and destruction of oligodendrocytes by JCV. The virus is ubiquitous and found worldwide. Human infection is usually acquired in the first decade of life and 80% to 90% of adults are seropositive. An asymptomatic persistent infection

occurs in kidney, lymphoid tissue, and CNS. Virus is shed into the urine, one study detected JCV DNA–positive urine in 40% of Caucasian adults in the United States (Agostini *et al*, 1997). In the majority of individuals, infection is kept under control by immune responses, though there is evidence that a more productive but asymptomatic infection occurs in some individuals, perhaps with mild disturbances to immune surveillance. PML is most frequently associated with leukemia, lymphoma, and particularly with immunosuppression.

PML has increased in significance over the last 20 years in parallel with the acquired immunodeficiency syndrome (AIDS) epidemic. Around 5% of AIDS patients develop PML and this accounts for an estimated 85% of all PML cases (Berger and Concha, 1995; Power *et al*, 2000). The combination generally results in a short survival time. JCV is detectable in peripheral blood in around 50% of human immunodeficiency virus (HIV)-seropositive individuals (Tornatore *et al*, 1992). Several studies have detected JCV DNA by polymerase chain reaction (PCR) in the brains of 'normal' individuals, that is, individuals without PML or HIV infection, with a frequency ranging from 12% to 50% (White *et al*, 1992; Elsner and Dorries, 1992; Ferrante *et al*, 1995; Gibson *et al*, 1993). However, these PCR-based studies have inevitably used human postmortem brain material and the extent to which positives represent infection of brain cells versus infection of leukocytes in the cerebral vascular has not been established. At present, it is unclear whether immunosuppression allows virus replication in the periphery with subsequent spread to the brain or whether JCV is generally present in the brain and immune responses lose control of this infection upon immunosuppression. In the case of PML in association with AIDS, there is also the issue of whether HIV activates JCV (Tada *et al*, 1990; Schmidbauer *et al*, 1990).

Clinically, PML has an insidious onset with dementia, weakness, visual disturbances, and sometimes ataxia. Disease is generally rapidly progressive. Neuropathology is characterized by both small foci and large areas of demyelination in several brain areas, including the cerebrum, brain stem, and cerebellum. Lesions are most frequently periventricular and occur at grey, white matter junctions. Large lesions are visible upon gross anatomical observation, can be necrotic, and contain few intact axons. Within small lesions, or around the edge of larger lesions, oligodendrocytes are enlarged and contain characteristic intranuclear inclusions. Electron microscopy demonstrates papovavirus particles in these nuclei and was the basis for the original association between PML and JCV (ZuRhein and Chou, 1965). These intranuclear crystalline arrays of virus are the basis for the intranuclear inclusions. The extent of mononuclear cell infiltration is variable. A prominent inflammatory response is often absent. However, plasma cells are generally present and numbers correlate with magnitude of intrathecal oligoclonal immunoglobulin G (IgG) reactive to the viral VP1 protein (Weber *et al*, 1997). Studies of immune responses in blood cells indicate an impairment of Th-1 immune responses, with presence of JCV-specific cytotoxic T lymphocytes, suggesting a more favorable prognosis (Weber *et al*, 2001). Demyelination in PML is generally accepted to result from direct destruction of virus infected oligodendrocytes (Astrom *et al*, 1958; Walker, 1978; Johnson, 1983).

Subacute sclerosing panencephalitis

SSPE is a human inflammatory demyelinating CNS disease that occurs several years after initial measles virus infection. It is predominantly a disease of children, with a prevalence of 1 in 100,000 to 1 in 1 million infected children. Measles infection at an early age, <2 years, is a risk factor (Modlin *et al*, 1977). The disease has an insidious onset with behavioral changes, mental deterioration, myoclonus, ataxia, and sometimes seizures and visual disturbances. Progress is variable, remissions may occur, but death usually results within 1 to 3 years. Serum and cerebrospinal fluid (CSF) have high or very high titers of measles virus antibodies (Connolly *et al*, 1967; Tourtellotte *et al*, 1968). Neurons and glial cells have cytoplasmic and nuclear inclusion bodies, immunostain for measles virus antigens, and contain paramyxovirus virions by electronmicroscopy (Bouteille *et al*, 1965; Jenis *et al*, 1973). Measles virus can be recovered from SSPE brains. Virus-infected cells can be widely distributed and most brain areas, with the possible exception of the cerebellum, can be infected. Neuropathology varies according to the duration of illness before death, though a picture of events can be constructed. Infiltrating mononuclear cells are first apparent in the meninges and perivascular cuffs and infiltrates can become extensive. There is activation of astrocytes and microglial cells, neuronophagia, and neuronal loss. Apoptosis has been observed both in infected and apparently uninfected neurons, oligodendrocytes, and microglial cells (McQuaid *et al*, 1997). Some infected neurons and oligodendrocytes contain fibrillary tangles similar to those seen in neurodegenerative diseases (McQuaid *et al*, 1994; Ikeda *et al*, 1995). Ultrastructurally, there have been many descriptions of virally infected neurons and oligodendrocytes, but there is general agreement that infected cells show no evidence of budding virus (Iwasaki and Koprowski, 1974; Dubois-Dalcq *et al*, 1974; Paula-Barbosa and Cruz, 1981; Schneider-Schaulies *et al*, 1995).

The events between initial measles virus infection and development at a later date, generally 6 to 8 years later, of SSPE are not clear. During acute infection, measles virus is present in leukocytes and these cells are also virus positive in SSPE (Fournier *et al*, 1985), but it is unclear how or when CNS

infection is established. CSF, electroencephalograms, and nuclear magnetic imaging (MRI) studies during acute measles virus infection suggest that this virus is more efficiently neuroinvasive (~30%) than clinical signs would suggest (Hanninen *et al*, 1980). Consistent with this, measles virus RNA has been detected in 'normal' human brain samples (Greenham *et al*, 1988). It is also unclear whether virus that infects the CNS is genetically identical to extraneural virus or whether neuroinvasion and/or development of disease requires generation and selection of specific genotypes. Analysis of measles virus sequences from SSPE brain samples indicates that, relative to reference strains, many genomes have mutations or deletions (Baczko *et al*, 1986; Cattaneo *et al*, 1988).

Expression of the viral envelope proteins M, F, and H is generally low or undetectable during persistent infection, whereas expression of the nucleocapsid (N) and phosphoprotein (P), which are components of the ribonucleocapsid and required for RNA replication, are always observed (Baczko *et al*, 1986; Liebert *et al*, 1986; Cattaneo *et al*, 1987). In common with other paramyxoviruses, measles virus demonstrates an attenuation of gene transcription from 3′ to 5′ along the genomic RNA in all cell types. This gradient is exaggerated in infected CNS cells, resulting in minimal transcription of 5′ genes (Schneider-Schaulies *et al*, 1989, 1995). Inhibition of translation of virus transcripts in some cell types has also been described (Schneider-Schaulies *et al*, 1989, Ogura *et al*, 1987, 1988; Miller and Carrigan, 1982; Yoshikawa and Yamanouchi, 1984). Interferon responses may be important and Mx protein is expressed in areas of infection in SSPE brains (Schneider-Schaulies *et al*, 1994, 1999).

There is a vigorous immune response in SSPE, with high levels of intrathecal virus-specific antibody, an intense mononuclear cell inflammatory response that includes $CD4^+$ and $CD8^+$ T cells, plasma cells, and macrophages, and proinflammatory cytokines (Nagano *et al*, 1991; Hofman *et al*, 1991). The antibody response is oligoclonal (Ebers *et al*, 1979; Hall *et al*, 1979). *In vitro*, the antibody is neutralizing and capable of complement-mediated lysis of infected human brain cells. Antibody can also decrease cell surface expression of viral glycoproteins on cultured cells and this has been suggested to contribute to the establishment of persistence *in vivo* (Joseph and Oldstone, 1975; Fujinami and Oldstone, 1980). Addition of neutralizing antibodies to persistently infected neural cells can down-regulate viral transcription (Barrett *et al*, 1985; Schneider-Schaulies *et al*, 1992). SSPE patients have no apparent immunological deficits (Mehta *et al*, 1994), nevertheless the immune responses generated are clearly unable to clear the persistent CNS infection; they may even be perpetuating it.

Understanding the exact mechanism of persistence and neuropathogenesis of measles virus infection and SSPE has been hampered by the lack of animal models. Natural virus isolates do not replicate in rodents, though rodent-adapted strains have been studied. In infected rat CNS cells, as observed *in vitro*, antibody enhances attenuation of transcription (Liebert *et al*, 1990). In mice, an active cellular immune system, particularly $CD4^+$ T cells, is important in the control of infection and can lead to resistance to encephalitis (Finke and Liebert, 1994; Finke *et al*, 1995; Urbanska *et al*, 1997). Two measles virus receptors have now been discovered, CD46 and CD150 (Naniche *et al*, 1993; Dorig *et al*, 1993; Tatsuo *et al*, 2000). CD46 transgenic mice have been generated and the course of CNS infection studied (Rall *et al*, 1997; Oldstone *et al*, 1999; Manchester and Rall, 2001; Patterson *et al*, 2001). Virus replicates predominantly in neurons; there is no attenuation of transcription but as in SSPE, there is little or no virus budding. Unfortunately, infection of oligodendrocytes is rare and there is no report that these mice develop lesions of demyelination.

In summary, the exact mechanism of demyelination in SSPE remains unclear. However, it is clear that virus infects and replicates in a restricted way in both neurons and oligodendrocytes, there are various mutations in the genomes of persistent viruses, there is loss of both cell types, and there is a strong immune response.

Mouse hepatitis virus (MHV)

In mice and rats, strains of MHV (Coronaviridae) cause a spectrum of disease from hepatitis and enteritis to encephalomyelitis and demyelination. MHV can infect and produce demyelination in the brain of nonhuman primates (Murray *et al*, 1992b). Human coronaviruses, related to MHV, have been detected in lesions of demyelination in MS brains (Murray *et al*, 1992a; Stewart *et al*, 1992). Neurotropic strains of MHV include MHV-4, MHV-JHM, and MHV-A59. These viruses are relatively virulent and demyelination is seen only in survivors of acute encephalitis. These animals often demonstrate hind limb paralysis and are sometimes tetraplegic. Modulation of infection and increased numbers of survivors can be obtained by passive administration of antisera (Buchmeier *et al*, 1984). However, a number of attenuated viruses, derived from these strains by mutagenesis or immune selection and maintain the ability to induce demyelinating disease, have been much studied to dissect the pathogenesis of this infection.

MHV strains are not efficiently neuroinvasive and neuropathology is generally studied following intracerebral inoculation, though intranasal inoculation is also an effective route of CNS infection (Pearce *et al*, 1994; Perlman *et al*, 1989). Differential ability in early replication and spread correlates with virulence (Fazakerley *et al*, 1992). Virulent strains are generally first observed in neurons, whereas avirulent strains infect neurons to a lesser extent. All strains infect

ependymal cells, astrocytes, oligodendrocytes, and microglia (Lavi *et al*, 1987; Sun and Perlman, 1995; Stohlman *et al*, 1995a). Virulent viruses target several brain areas but infection of and damage to structures in the limbic and olfactory systems is common (Lavi *et al*, 1986, 1990; Fazakerley *et al*, 1992; Sun and Perlman, 1995). Tracking of virus through olfactory system pathways is observed following intranasal inoculation (Lavi *et al*, 1986; Perlman *et al*, 1989; Pearce *et al*, 1994; Lane *et al*, 1997). Spread of the virus is along neural pathways (Perlman *et al*, 1989; Fazakerley *et al*, 1992). The virus envelope spike glycoprotein S is a major determinant of virulence, demonstrates great variability, and is responsible for virus binding and entry. One receptor for the virus is biliary glycoprotein 1a, a member of the carcinoembryonic antigen family; other members of this family may also function as receptors (Williams *et al*, 1991; Dveksler *et al*, 1991; Chen *et al*, 1995). SJL mice have a polymorphism of this gene, which renders them resistant to widespread MHV infection (Dveksler *et al*, 1993). Antibodies, $CD4^+$ and $CD8^+$ T cells can each provide protective responses (Buchmeier *et al*, 1984; Stohlman *et al*, 1986; Yamaguchi *et al*, 1991). Differences in immune responses are important in determining the outcome of infection. For example, following MHV-JHM infection, BN rats have small nodular plaques of CNS demyelination and remain clinically healthy, whereas Lewis rats have large areas of white matter inflammation and develop acute encephalitis or subacute demyelinating disease (Watanabe *et al*, 1987). In BN rats, spread of infection is controlled by a local antibody response and the characteristically small lesions contain many plasma cells. In Lewis rats, plasma cells are rarely observed and a strong cell-mediated immune response develops, giving rise to large areas of white matter inflammation (Dorries *et al*, 1987).

In mice surviving intracerebral inoculation, infectious virus is generally detectable in the CNS for at least the first week but is undetectable by 2 weeks. Virus clearance is mediated principally by $CD8^+$ T lymphocytes, but this is not a sterile immunity and virus can persist (Stohlman *et al*, 1995a). Perforin-mediated cytotoxicity controls infection of astrocytes and microglia, whereas interferon-γ controls infection of oligodendrocytes (Lin *et al*, 1998b; Parra *et al*, 1999). This dichotomy is interesting as major histocompatability complex (MHC)-I is expressed on each of these cell types (Redwine *et al*, 2001). Antibody responses are crucial in controlling spread of virus (Ramakrishna *et al*, 2002). Persistence of viral RNA and protein has been observed, mostly in glial cells including oligodendrocytes, for up to a year post infection (Knobler *et al*, 1982; Lavi *et al*, 1984; Perlman and Ries, 1987; Adami *et al*, 1995; Parra *et al*, 1999; Fleming *et al*, 1995). Analysis of virus RNA during persistence indicates that many viral genomes have mutations, particularly point and deletion mutations in the S and N genes (Adami *et al*, 1995). These genomes could be defective and unable to generate infectious virus. Persistence of a genotype with changes in a defined CD8 T-cell epitope have also been observed, consistent with selection of immune escape variants (Pewe *et al*, 1996).

Demyelination is a feature of the neuropathology in mice surviving infection with virulent strains as well as in mice infected with avirulent strains. Following inoculation of mice with MHV 2.2v-1, infection of oligodendrocytes and demyelination are observed within the first week (Wang *et al*, 1992). Demyelination increases over the following 2 weeks and is followed by a period of remyelination and repair. However, while this is ongoing, new focal lesions are initiated (Erlich *et al*, 1987). Most studies describe demyelination concomitant with or following clearance of virus infectivity and lesions have been described months post infection (Woyciechowska *et al*, 1984; Haspel *et al*, 1978; Stohlman and Weiner, 1981; Herndon *et al*, 1975; Erlich *et al*, 1987). Demyelinating lesions are predominantly primary demyelination with axonal sparing and are generally inflammatory with scattered lymphocytes and lipid containing macrophages (Lavi *et al*, 1986; Lampert *et al*, 1973; Stohlman and Weiner, 1981). Demyelination is generally followed by remyelination, which can be seen first a few weeks after onset of demyelination (Takahashi *et al*, 1987; Kristensson and Norrby, 1986; Herndon *et al*, 1977).

That demyelination is sometimes observed as early as the first week post infection, when virus titers are high, might suggest a direct viral pathology. In the Lewis rat, early after infection, death of JHM-infected oligodendrocytes is necrotic, whereas death of apparently uninfected oligodendrocytes and oligodendrocyte death in the chronic phase of disease after virus clearance is apoptotic (Barac-Latas *et al*, 1997). This could be consistent with an early direct viral effect and a later bystander effect mediated by inflammatory responses. Whereas there may be some early direct viral destruction of oligodendrocytes, demyelination is described as absent, rare, or reduced in SCID, RAG1, or immunosuppressed mice, indicating that a large element of the demyelination requires immune or inflammatory responses. SCID mice neither clear infectious brain virus nor display large lesions of demyelination, whereas athymic *nu/nu* mice fail to clear virus but do have lesions of demyelination (Houtman and Fleming, 1996). RAG1-null mice have been described to have no (Wu *et al*, 2000), small (Wu and Perlman, 1999) or moderate (Matthews *et al*, 2002) lesions of demyelination. In the case of RAG1-null mice with no demyelination, transfer of $CD4^+$ or $CD8^+$ T cell–enriched lymphocyte populations resulted in demyelination, with greater macrophage infiltration into CNS lesions and greater clinical disease following $CD4^+$ cell transfer, but greater spinal cord demyelination following $CD8^+$ cell transfer, suggesting that $CD4^+$ and $CD8^+$ T cells are both capable of mediating lesions

of demyelination (Wu *et al*, 2000; Wu and Perlman, 1999). Consistent with this, other studies demonstrate that neither $CD4^+$ nor $CD8^+$ T cells alone are required for demyelination because lesions develop in mice deficient in β-2-microglobulin, MHC class II, CD4, or CD8 or in mice depleted of $CD8^+$ T cells (Houtman and Fleming, 1996; Gombold *et al*, 1995; Lavi *et al*, 1995, 1999; Lane *et al*, 2000). Depletion of CXCL10 (IP-10) or RANTES reduces inflammation and demyelination (Lane *et al*, 1998, 2000; Liu *et al*, 2001). These chemokines probably mediate their effect by recruiting T cells and macrophages. However, depletion of blood macrophages does not reduce demyelination (Xue *et al*, 1999). CXCL10 suppression of inflammation early after infection also delays CNS virus clearance and increases mortality (Liu *et al*, 2000). However, neutralization of CXCL10 in mice with established demyelinating disease inhibits progression of demyelination, increases remyelination, and improves neurologic function (Liu *et al*, 2001). Neither perforin, Fas, tumor necrosis factor (TNFα), interleukin (IL)-10, interferon-γ, nor immunoglobulin are required for demyelination (Lin *et al*, 1997, 1998a; Parra *et al*, 1999, 2000; Stohlman *et al*, 1995b; Matthews *et al*, 2002). In rats, JHM infection generates autoreactive T cells capable of mediating brain inflammation on adoptive transfer (Watanabe *et al*, 1983). Whether these contribute to the pathology or are also present in mice remains unclear. In humans, T-cell lines cross-reacting with myelin basic protein and human coronavirus 229E could be established from some (4/16) multiple sclerosis patients but not controls (Talbot *et al*, 1996).

In summary, the MHV model system demonstrates that despite an active immune response, virus is able to persist in glial cells, predominantly astrocytes but including oligodendrocytes. Persistence is associated with changes in the virus genome, possibly resulting in defective virus or virus that escapes immune surveillance. Persistence results in lesions of demyelination. Some demyelination may be due to direct virus infection and destruction of oligodendrocytes, but the majority is dependent upon specific immune and inflammatory responses.

Theiler's murine encephalomyelitis virus (TMEV)

TMEV (Picornaviridae) was first isolated from mice presenting with a flaccid paralysis of the hind limbs (Theiler, 1934). TMEV is a natural enteric pathogen of mice that is transmitted by the feco-oral route. Based on differing biological characteristics, strains of TMEV are generally divided into two subgroups. Following intracerebral inoculation, the neurovirulent GDVII and FA strains rapidly infect neurons and induce an acute and usually fatal encephalomyelitis. The second group, known as Theiler's original (TO), includes the BeAn, DA, WW, and Yale strains and causes a biphasic disease in some strains of mice (Lipton, 1975). During the first phase, lasting for approximately 2 weeks, virus preferentially infects neurons within the gray matter areas of the brain and spinal cord and disease may present clinically with a transient hind limb paralysis. By 3 weeks post infection, despite clearance of virus from the initial infected gray matter areas, the second phase of disease occurs. This is characterized by persistent and long-lasting infection of cells within the white matter of the spinal cord accompanied by chronic inflammation and demyelination. Virus persistence is necessary for inducing the demyelinating disease and determinants influencing susceptibility or resistance to demyelinating disease are polygenic. The strongest genetic influence involves the immune response genes conferred by the MHC H-2 haplotypes, in particular H-2D (Rodriguez and David, 1985; Clatch *et al*, 1985; Azoulay-Cayla *et al*, 2001). Other susceptibility loci include *Tmevd-1* on chromosome 6, which maps close to genes coding for the T-cell receptor (TCR) β chain (Melvold *et al*, 1987), *Tmevd-2* on chromosome 3 (Melvold *et al*, 1990), and a locus mapping close to the *Ifng* locus on chromosome 10 (Bureau *et al*, 1993).

That resistance to persistent infection is dominantly associated with MHC class I genes suggests that $CD8^+$ T cells are important determinants of virus persistence and demyelination. Depletion of $CD8^+$ T cells or infection of β2-microglobulin–deficient mice, on a resistant background, results in impaired ability to clear virus and development of demyelinating disease (Borrow *et al*, 1992; Fiette *et al*, 1993; Pullen *et al*, 1993; Rodriguez *et al*, 1993). Generation of a strong virus-specific $CD8^+$ cytotoxic T lymphocyte (CTL) response early in infection may confer resistance to subsequent development of demyelinating disease. Splenocytes from resistant C57BL/6 mice mount a faster and stronger anti-TMEV CTL response than splenocytes from susceptible SJL/J mice (Dethlefs *et al*, 1997). However, a recent study demonstrated within the CNS of TMEV-infected SJL/J mice $CD8^+$ T cells against a dominant VP3 virus capsid epitope (Kang *et al*, 2002). These were H-$2K^s$ restricted and although H-2K molecules can present viral peptides to CTL in susceptible SJL/J mice, a low level of H-2K expression on cells within the CNS or a preferential expression of H-$2D^s$ (Altintas *et al*, 1993) may result in inefficient antigen presentation and diminished CTL reactivity.

Although the role of $CD8^+$ T cells in the first phase of the biphasic disease appears critical in controlling infection, their role in promoting demyelination is controversial. In mice deficient in β2-microglobulin (Fiette *et al*, 1993) or perforin (Palma *et al*, 2001; Rossi *et al*, 1998), susceptibility to demyelinating disease is exacerbated, suggesting no requirement for $CD8^+$ T cells. However, other studies suggest that despite increased demyelination in β2-microglobulin–deficient mice (Rodriguez *et al*, 1993), $CD8^+$ T cells

are necessary for the perforin-dependent development of neurologic deficits (Murray *et al*, 1998), as demyelination alone is insufficient to cause clinical symptoms (Rivera-Quinones *et al*, 1998). Yet, other studies demonstrate that mice depleted of $CD8^+$ T cells present with aggravated demyelination, enhanced inflammatory Th-1 cell responses, and earlier onset of clinical disease (Begolka *et al*, 2001), suggesting that $CD8^+$ T cells may be necessary to downregulate the proliferation of virus-specific $CD4^+$ T-cell responses (Filaci *et al*, 2002).

Despite the ongoing presence of antiviral immune responses in susceptible mouse strains, TMEV can persist within the spinal cord for long periods (Trottier *et al*, 2002). Within the spinal cords of persistently infected mice, several studies suggest that macrophages harbor the greatest viral load (Lipton *et al*, 1995; Bihl *et al*, 1997) and to a lesser extent glial cells (Brahic *et al*, 1981), including astrocytes (Aubert *et al*, 1987) and oligodendrocytes (Rodriguez *et al*, 1983; Simas and Fazakerley, 1996). A recent study using brain-derived primary cell cultures suggests that high levels of virus antigen in microglia may represent mainly phagocytosed virus particles and that preferential replication in astrocytes and oligodendrocytes may be the basis of virus persistence (Zheng *et al*, 2001).

Initial damage to the myelin-producing oligodendrocytes may result from direct cytolysis of virally infected cells. However, because most studies suggest that only low numbers of oligodendrocytes are infected, it is more likely that oligodendrocyte damage results from exposure to immune responses. Many studies strongly suggest that the demyelinating phase of the disease is principally dependent upon the generation of anti-TMEV–specific class II restricted $CD4^+$ T-cell responses (Clatch *et al*, 1987). Flow cytometry studies indicate that the majority of activated T cells infiltrating the CNS are $CD4^+$ (Pope *et al*, 1996). Depletion of $CD4^+$ T cells abrogates demyelination whereas transfer of TMEV-specific $CD4^+$ T cells enhances disease (Borrow *et al*, 1992; Gerety *et al*, 1994). Initial $CD4^+$ T-cell responses are virus specific and particularly directed against an immunodominant VP2 epitope (Gerety *et al*, 1991); however, subsequent T-cell responses against myelin components, including proteolipid protein (PLP), are observed 3 to 4 weeks after onset of clinical disease. As disease progresses, "epitope spreading" is observed, with additional delayed-type hypersensitivity (DTH) responses to multiple myelin antigens, including myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG) (Miller *et al*, 1997; Katz-Levy *et al*, 1999). Because T-cell reactivity to virus develops in advance of T-cell reactivity to myelin epitopes, molecular mimicry seems unlikely. Whether or not these later, anti-myelin responses are involved in propagating the demyelinating disease or are a consequence of it remains unclear. Induction of tolerance to TMEV and CNS epitopes can inhibit subsequent development of demyelinating disease (Karpus *et al*, 1995; Neville *et al*, 2002).

In summary, the TMEV model system demonstrates how genetics can control susceptibility to a persistent CNS virus and the importance of early, particularly $CD8^+$ T-lymphocyte, immune responses in determining the balance between virus elimination and persistence. It also establishes the important concept that CNS virus persistence can generate an autoimmune response, though the relevance of this to the pathogenesis of demyelination remains to be determined.

Semliki Forest virus (SFV)

The most commonly studied strains of SFV (Togaviridae) are L10, prototypes A7 and A7(74), which are respectively virulent and avirulent in adult mice. A major determinant of virulence resides in the nsP3 protein, a component of the viral replicase (Tuittila *et al*, 2000). All SFV strains are virulent in neonatal and suckling mice by all routes of inoculation. A number of viruses generated by mutagenesis have also been studied; from the point of view of demyelinating disease, the most notable of these is M9 (Barrett *et al*, 1980). SFV has the advantage of being neuroinvasive as well as neurotropic, allowing study of virus entry into the CNS and the functioning of the blood-brain barrier. Following intraperitoneal infection, all strains replicate in muscles and other tissues, giving rise to a plasma viremia (Pusztai *et al*, 1971). Virus crosses the cerebral endothelial cells to initiate perivascular infection of CNS cells, predominantly neurons and oligodendrocytes (Fazakerley *et al*, 1993; Balluz *et al*, 1993). In neonatal mice or adult mice infected with virulent strains, virus spreads rapidly along neurites, giving rise to a widespread infection that is lethal within a few days. In contrast, in adult mice infected with avirulent strains, once virus reaches the CNS, it remains confined to small perivascular foci around the initial sites of entry (Fazakerley *et al*, 1993). In the absence of T-cell immunity, this pattern of scattered focal infection persists, probably for life, with no clinically apparent deficit (Fazakerley and Webb, 1987; Amor *et al*, 1996).

SFV-induced CNS demyelinating disease has been most studied following infection of adult mice with the A7(74) strain of virus. Following intraperitoneal infection of immunocompetent adult mice, infectious virus is detectable in the brain by 24 h. Titers rise thereafter but are rapidly reduced following the onset of immune responses. By infectivity assay, virus is detectable in the brain for the first 8 days but viral RNA is detectable for longer periods by *in situ* hybridization (Fazakerley *et al*, 1993). Reverse transcriptase (RT)-PCR studies following M9 infection indicate that viral RNA can be detected in the brain for even long periods, >90 days (Donnelly *et al*, 1997),

and following A7(74) infection, antibody-producing plasma cells in the brain and intrathecal antiviral antibody are detectable for months, suggesting persistence of viral antigen (Parsons and Webb, 1984, 1989). Disturbance of the blood-brain barrier is apparent between 4 and 10 days, allowing passage of serum proteins, including immunoglobulins (Parsons and Webb, 1982; SoiluHanninen *et al*, 1994). This leaky barrier corresponds to the time of increasing inflammatory cell infiltration and reduction of brain virus titers and could be related to the massive influx of cells or cytokine-mediated effects. Cerebral endothelial cells up-regulate adhesion molecule expression and MHC-I expression is up-regulated on a concentric network of parenchymal cells with a dendritic morphology, most probably microglial cells, around the foci of infection (SoiluHanninen *et al*, 1997; Morris *et al*, 1997). Pleocytosis is apparent from 4 days and subsides by 9 days, but cell counts in the cerebrospinal fluid do not return to normal for weeks (Parsons and Webb, 1984). An intense inflammatory response characterized by perivascular cuffing, with invading cells spreading into the parenchyma, is apparent histologically from 3 days. Demyelination is first apparent by luxol fast blue staining of paraffin sections around 14 days post infection (Figure 1), but small focal lesions are apparent by electron microscopy at 10 days (Suckling *et al*, 1978; Kelly *et al*, 1982). In the optic nerve, there are lesions of demyelination and changes in visually evoked responses and axonal transport (Tremain and Ikeda, 1983). These are features that also occur in MS. No demyelination is apparent in SCID mice, athymic mice, or mice depleted of $CD8^+$ T cells (Amor *et al*, 1996; Fazakerley *et al*, 1983; Subak-Sharpe *et al*, 1993). Whether $CD8^+$ T cells are required to directly destroy, possibly by perforin- or Fas-mediated pathways, virally infected oligodendrocytes or whether they act via other mechanisms, such as release of interferon-γ remains to be studied.

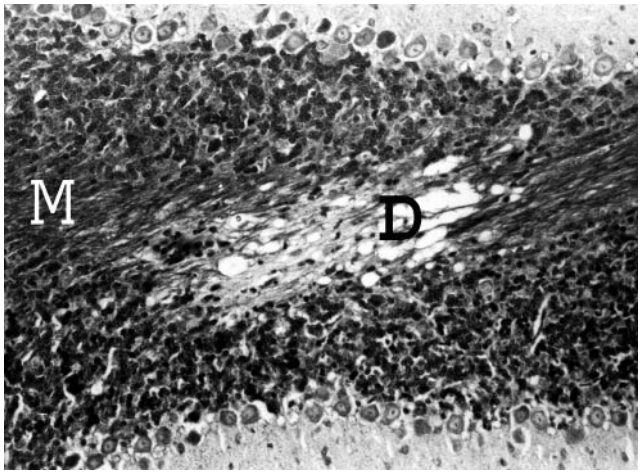

Figure 1 A lesion of demyelination in the white matter of the cerebellum 18 days after SFV A7(74) infection. Stained with luxol fast blue and cresyl violet. M indicates normal myelin and D the area of demyelination that is vacuolated and contains inflammatory cells.

In summary, the SFV model allows for the study of the kinetics of a natural infection that is highly neuroinvasive and is associated with changes in the blood-brain barrier. Demyelination in SFV infection is immune mediated and appears to result from a $CD8^+$ T cell–mediated destruction of virally infected oligodendrocytes, with little or no direct viral destruction of these cells. This model also demonstrates changes in optic nerves similar to those seen in MS.

Consistent features and questions

Demyelination in PML seems simply to result from virus infection and destruction of oligodendrocytes in individuals where immune responses are no longer able to control this common infection. The situation may be exacerbated by molecular interactions between JCV and HIV in AIDS patients. In SSPE, measles virus infects glial cells but cannot be eliminated from the brain despite strong immune responses. It is notable that measles virus persistence is associated with restricted virus replication and that budding virions cannot normally be seen. If there are any common features in the rodent model systems, they are that the virus can infect and persist in glial cells, that immune responses develop but fail to eradicate the infection, that $CD8^+$ T cells are particularly important in early virus clearance, that in the absence of virus eradication, antibodies prevent further virus spread, and that although demyelination may to a limited extent be the result of direct virus destruction of oligodendrocytes, it is for the most part the consequence of immune and inflammatory responses. On top of this can be built up further layers of variables that govern whether or not infection will lead to demyelinating disease. The first is age, as many neurotropic infections show age-related differences (Fazakerley, 2001). The second is the nature of the infection, the dose, and the strain of virus. Some viruses are efficiently neuroinvasive whereas others are not. With many viruses that can cause CNS disease, invasion of the CNS may be dependent upon other aspects of physiology, such as diet, stress, or intercurrent infection, or this process may be stochastic. The timing of neuroinvasion relative to development of immune responses is important, and the virus, its strain, and the level of immunity determines the extent of CNS infection. A third individual variable is host genetics, which determines the type and magnitude of immune response and in turn governs susceptibility to persistent infection and disease (Brahic and Bureau, 1998).

Further understanding is needed in all of the above areas but perhaps particularly to the question why it is that these and several other viruses are able to persist in CNS cells. Certainly, many of the viruses that

persist in the CNS do not readily persist in other organ systems. In the examples discussed here, it is persistence in glial cells that is crucial, but there are several viruses that can persist in neurons. Is it something about immune responses in the specialized environment of the CNS, perhaps an inability to eliminate virus-infected cells or a switching off of immune response at too early a time, or is it something fundamental about the relationship between viruses and long-lived neural cells?

Restricted infection of neural cells is a feature of SSPE, of measles, of SFV A7(74) and TMEV BeAn in the mouse models described above, and also of lymphocytic choriomeningitis virus infection (Oldstone and Buchmeier, 1982; DeLaTorre *et al*, 1993). The nature of these persistent, restricted infections remains unclear. One possibility is that individual cells are infected for long periods of time, though most results could also be explained by a dynamic persistence in which infection of an individual cell has a given half-life and that with time viral material is eliminated, with or without cell loss. Infection of new cells could be initiated at a slow rate, possibly in some cases by spread between cells without formation of virus particles, as has been described for measles virus (Lawrence *et al*, 2000). Whether there is gradual cell loss remains unclear, as careful measurements have not been done. The extent of neuronal loss in the brains of mice infected with scrapie was not appreciated until precise quantitation was done (Jeffrey *et al*, 1995). So the extent of cell loss with time remains unclear as does the nature of persistence, which could be static, that is, fixed within individual cells, or dynamic, with movement between cells. In these restricted infections, replication at some level is blocked. This could be a permanent or a temporary block and it could vary between cell types or individual cells. A temporary block could result from inhibitory factors, for example interferons, and remain until these drop below an effective level. There is, for example, evidence of interferon-activated responses in measles virus–infected areas of SSPE brains (Schneider-Schaulies *et al*, 1999). Alternatively, cellular factors could be required for productive replication, perhaps produced rarely in response to cell activation. A situation in which infection is generally restricted but occasionally productive could occur. Restricted infection could also result from infection of cells with specific virus genotypes that have lost the ability to undergo productive infection in specific cell types. The genotypes of JCV, measles, and MHV that persist in the brain are different to the original infecting viruses. It is generally not clear whether these genetic changes are required to establish persistence or are a consequence of persistence. The latter seems most likely.

It is generally considered that upon infection, most virus-infected cells initiate apoptosis as an altruistic response to curtail virus spread. This makes evolutionary sense in renewable cell populations but not in the specialized, postmitotic environment of the CNS, and it seems that virus-infected mature CNS cells are more refractory to initiating apoptosis than cells in other tissue systems (Allsopp and Fazakerley, 2000). This relative resistance to apoptosis is probably one major factor in allowing many viruses to persist in CNS cells. Another major factor is the failure of immune responses to establish sterile immunity. $CD8^+$ T lymphocytes seem to be important in controlling CNS virus infections at early time points and may function by eliminating virus-infected cells. If these are oligodendrocytes, this will contribute to the demyelination. Certainly, $CD8^+$ T cells seem to contribute to demyelination in MHV and SFV infections (Subak-Sharpe *et al*, 1993; Wu *et al*, 2000). Why early immune responses do not progress to completely eradicate the infection is not clear. At least in the case of the Theiler's virus, inability to clear the infection, persistence, and subsequent demyelination are determined by host genetics (Brahic and Bureau, 1998). Perhaps in addition, cells with a restricted virus infection are not targeted by immune responses, do not provide signals sufficient for immune-mediated destruction, or do so only with time. In Theiler's virus and probably to an extent in MHV infections, this persistence drives a chronic immune response leading to release of toxic factors that mediate bystander damage, loss of uninfected oligodendrocytes, and generation of lesions of demyelination. In the case of at least Theiler's virus, this damage eventually leads to the release of CNS antigens, epitope spreading, and generation of autoimmune responses (Miller *et al*, 1997).

Multiple sclerosis

It seems appropriate to conclude with some reference to MS because it was the lack of understanding of the etiology and pathogenesis of this disease that initiated study of animal models of CNS inflammation and demyelination. MS is the most common human demyelinating disease of the CNS, affecting around 2.5 million people, with an incidence of 7 per 100,000 per year, a prevalence of 120 per 100,000, and a lifetime risk of 1 in 400 (Compston and Coles, 2002). Prevalence varies according to genetics, latitude, and other factors, but reaches 300 per 100,000 in areas of Northern Europe. MS is prevalent amongst Caucasians but rare in Africans or Asians. Even within areas of high risk, these ethnic populations are at a lower risk than Caucasians. In most patients (80%), the disease is characterized by acute relapses with remissions, followed by a secondary progressive phase. For 20% of patients, the disease is progressive from the onset (primary progressive). Pathologically, MS manifests as acute focal inflammatory demyelination, with varying degrees of axonal loss, culminating in development of multifocal sclerotic plaques. The extent of cellular infiltration in acute lesions and the degree of oligodendrocyte

loss is highly heterogeneous and may reflect distinct pathogenic mechanisms (Lucchinetti *et al*, 2000).

The etiology of MS remains unknown but involves the interaction of genetic, immunological, and probably environmental factors. Although the risk of developing the disease is significantly higher in family members of patients with MS (Ebers *et al*, 1995), genetic predisposition is polygenic (Oksenberg *et al*, 2001). Among identified susceptibility loci, the HLA class II alleles DR15 and DQ6 (DRB1*1501 and DQB2*0602) show the strongest association in Caucasians (Olerup and Hillert, 1991). However, a concordance rate of only 30% amongst monozygotic twins indicates the involvement of nongenetic factors (Ebers *et al*, 1986). Evidence for an environmental element is suggested by migration studies wherein movement before adolescence from a high-risk to a low-risk region reduces the risk of developing MS (Gale and Martyn, 1995). Equally, several "MS epidemics," frequently amongst island populations, have supported the role for an environmental agent. Within the Faroe Islands, documented cases of MS only occurred in the years following the occupation of the Islands by British troops during World War II (Kurtzke *et al*, 1993). Associations between infectious agents and MS, including measles virus (Field *et al*, 1972), parainfluenza virus (ter Meulen *et al*, 1972), canine distemper (Cook and Dowling, 1977), *Chlamydia pneumoniae* (Sriram *et al*, 1999), Epstein-Barr virus (Ascherio *et al*, 2001), human herpes virus-6 (Challoner *et al*, 1995) and retroviruses (Perron *et al*, 1997), have been reported. Nevertheless, to date, no specific environmental agent has been substantiated as the etiologic agent of this disease.

Whereas the initiating event in the etiology of MS remains unknown, a role for the immune system in the pathogenesis is clear. One of the diagnostic markers for MS is the presence of an intrathecal oligoclonal IgG response. Clonal expansion of T cells, primarily of the $CD8^+$ phenotype, has been reported in CNS lesions (Babbe *et al*, 2000; Jacobsen *et al*, 2002). Exacerbation of disease following treatment with IFN-γ suggests that $CD4^+$ T cells with a Th-1 phenotype might be important in promoting MS (Panitch *et al*, 1987). However, in clinical trials, antibodies against $CD4^+$ T cells (van Oosten *et al*, 1997) or anti-TNFα antibodies (van Oosten *et al*, 1996) fail to ameliorate disease.

Several hypotheses have been promoted to explain the pathogenesis of MS but two are particularly plausible. Firstly, the autoimmune hypothesis, wherein disease in mediated by autoreactive T cells specific for components of the myelin sheath. Secondly, the infectious hypothesis, where a pathogen infects the CNS and persists, driving damaging inflammatory responses. Both hypotheses would include that disease would only develop in genetically susceptible individuals. These two hypotheses are not mutually exclusive. Damage resulting from a CNS virus infection can lead to epitope spreading of immune specificities to generate autoimmune responses (Miller *et al*, 1997). It has also been suggested that autoimmunity could arise as a result of cross-reactivity between viral and myelin antigens (Oldstone, 1987; Wucherpfennig and Strominger, 1995). It is, however, disappointing that despite decades of intensive research, the etiology of MS has not been determined. In support of a viral etiology, which is often forgotten by researchers studying autoimmunity, it should be remembered that virtually all demyelinating diseases of man and animals of known etiology are viral, there is no *a priori* reason to consider that MS should be any different and there remains no really convincing evidence that MS is a spontaneously arising autoimmune disease. A description of inflammatory lesions and genetic susceptibility are entirely consistent with an infectious etiology and specificity for autoantigens does not preclude an originating infection, nor does it indicate that these specificities are pathogenic. That many MS lesions have a preponderance of $CD8^+$ lymphocytes is consistent with viral-driven responses. CSF oligoclonal IgG bands are found both in MS and following CNS virus infections. The recent revaluation of axonal loss in MS lesions indicates that this can be a major feature (Kornek *et al*, 2000; Lucchinetti *et al*, 2000), and in the viral models, there are varying degrees of axonal loss. Finally, would we expect to find an infectious agent in all cases of this disease, even many years after onset? In support of a virus etiology for MS, it should be remembered that absence of evidence is not evidence of absence and MS may yet turn out to be the most important example of virus-associated demyelinating disease.

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