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Virological and pathological processes involved in Theiler's virus infection of the central nervous system

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Theiler's virus strains GDVII and FA cause an acute encephalitis when injected intracerebrally into mice, whereas strains To, BeAn and DA establish a persistent infection and produce a chronic demyelinating disease. The chronic infection is also dependent on the mouse strain used, with susceptibility linked in part to the D locus of the MHC. The region of the virus genome associated with neurovirulence maps to the P1 region, encoding the capsid proteins, and to the 5' non-coding region. There is evidence that BeAn/DA virus persists in oligodendrocytes, where it reactivates to initiate demyelinating disease. Host factors are involved in the development of the lesion, including CD4⁺ T cell responses. These lymphocytes most probably mediate damage through activation of macrophages leading to local destruction of glial cells. Another possible pathological role for the immune system is the recognition of nerve cell antigens and the initiation of autoimmune disease. Such a virus-triggered phenomenon may well underlie human CNS diseases such as multiple sclerosis.

Key words: Theiler's virus / demyelination / autoimmunity / CD4 T cells

IN 1933, Max Theiler reported the isolation of a picornavirus from the nervous system of mice with flaccid paralysis.¹ The virus, referred to as Theiler's virus or Theiler's murine encephalomyelitis virus (TMEV), transferred the paralytic disease to laboratory mice. An acute infection occurred in motor neurons of the spinal cord and the resulting disease was similar to human poliomyelitis. Indeed, in the 1950s, Theiler's virus infection was used as a model for this debilitating human CNS disease. In the following years, additional isolates were identified which produced a chronic infection of the CNS resulting in a primary inflammatory demyelinating disease.² The infected animals undergo episodes of demyelination and remyelination with lesions confined to the white matter of the spinal cord. In some strains

of mice the infection was observed to undergo a biphasic course.³ The acute stage lasts for 3-4 weeks and involves infection of motor neurons in the grey matter. Animals surviving this stage progress toward the chronic infection and demyelination. This stage of infection shows similarities with multiple sclerosis (for reviews see refs 4,17,42).

The virus

Theiler's virus is a member of the cardiovirus genus of the picornaviridae. These viruses are non-enveloped, possessing a single-stranded RNA genome of positive sense and encapsidated within a 60-subunit protein shell, 20-30 nm in diameter. The genome contains a 3' polyadenylated tail and a large 5' non-coding region of 1064 nucleotides.⁵ Translation yields a polyprotein, which is cleaved by viral proteases to produce capsid proteins and enzymes involved in replication.

Isolates of the virus fall into two groups: virulent strains (GDVII and FA) which produce a rapid and fatal encephalitis when injected intracerebrally, and persistent or avirulent strains (To, BeAn, DA), which are associated with a biphasic disease resulting in a chronic demyelination. Other phenotypic differences are summarised in Table 1. The genomes of both types of virus have been sequenced and show a great deal of homology: 95% at the genome and 90% at the protein level.^{5,6} As the strains have different biological properties, interest has centred on mapping the regions of the genome associated with neurovirulence and persistence. Essentially, two approaches have been used: the generation of chimaeric viruses containing genomic mixtures of the two virus groups: and the generation or isolation of mutant strains which have reverted to an avirulent phenotype. In this case any changes in nucleotide sequence of the mutant virus can be compared with that of the parent and hence the region conferring the avirulent phenotype identified.

Using chimaeric viruses has shown that the L/P1(coding for capsid proteins)/2A region of the genome appears greatly to influence the phenotype of the new virus.^{7,43} For example, replacing the

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Table 1. Comparison of the To and GDVII Theiler's virus subgroups

	To/BeAn/DA	GDVII/FA
Plaque size	1 mm	5 mm
Log ₁₀ pfu LD ₅₀ (i.c.)	4.0	1.0
Type of disease <i>in vivo</i>	Chronic inflammatory demyelination	Acute encephalitis
Cell types infected	Neurons, glial cells, macrophages	Neurons
Persistence <i>in vivo</i>	Yes	No
Infection <i>in vitro</i>	Membrane-associated virus	paracrystalline arrays of virus

GDVII (virulent) L/P1/2A region with that of BeAn (avirulent) renders the new virus avirulent, with the ability to persist *in vivo* and produce demyelinating disease. Within this region of the genome, the VP1 protein (including 27 amino acids of 2A) appears to have a strong influence on the disease phenotype of the virus.⁴³ The importance of the VP1 region is further highlighted by the isolation of an antibody escape mutant with a single amino acid change in VP1.⁸ This altered DA virus was considerably more avirulent than the parent.

The 5' non-coding region of BeAn also influences the phenotype of the virus, converting GDVII into an avirulent virus.⁷ This observation is in line with other studies showing that the 5' non-coding region of poliovirus type 3⁹ and Mengo virus¹⁰ (a cardiovirus with a strong homology to Theiler's virus) are strongly associated with neurovirulence.

The infection

The nature of the infection depends not only on the strain of virus but also the genotype of the mouse. Whereas all mouse strains investigated are susceptible to the GDVII strain and produce encephalitis, only certain mouse strains are permissive for the avirulent strains (BeAn and DA). For example, a comparison of different mouse strains infected intracerebrally (i.c.) with 10⁴ plaque-forming units (pfu) of BeAn resulted in 100% of SJL, 70% of CBA and 0-10% of Balb/c mice afflicted with demyelinating disease.^{11,29,30,36} Another important factor in the development of the chronic demyelinating disease is the age of animal at the time of infection. The optimum age is 4-5 weeks; beyond this, animals become increasingly resistant to the virus.

The acute infection of mice with GDVII is invariably fatal, i.e. 1-10 pfu injected i.c. is sufficient

to produce 50% mortality in mice on various inbred and outbred backgrounds.¹² The virus predominantly infects motor neurons in the cerebral cortex and the ventral horns of the spinal cord. An inflammatory infiltrate of mononuclear cells accompanies the infection and the resulting encephalitis and cytolysis of neurons leads to death in 5-7 days. The entry of virus into the CNS is principally by axonal transport.¹³ This is clearly demonstrated by injecting GDVII virus intramuscularly, when only motor neurons innervating the site of infection are destroyed resulting in paralysis (Blakemore, personal communication). Other portals of entry into the CNS could include endothelial cells at the blood-brain barrier, where viral RNA has been identified by *in situ* hybridisation.¹⁴

Infection of mice with avirulent Theiler's virus strains involves a wider cell tropism, with the virus persisting in the host and triggering demyelinating disease.¹⁵ As a typical example, I shall consider i.c. infection of CBA mice with 10⁴ pfu of BeAn.¹⁶ Infectious virus can be isolated from the brain and spinal cord in high titres for up to 4 weeks. Other tissues, e.g. heart and spleen, may become infected during the first 10 days. In the CNS, virus is associated with neurons, glial cells and macrophages, and paralytic disease may result in 10% of the animals. Approximately 70% of mice surviving the acute infection will progress towards a chronic demyelinating disease. Early in the chronic phase most mice show no signs of CNS disease and have no detectable virus in the cord or brain, but animals showing signs of CNS pathology have infectious virus associated with demyelinating lesions.¹⁷ This suggests that virus is the initiator of the disease process, probably as a result of infecting and destroying oligodendrocytes. Evidence for the involvement of virus in this process comes from various sources. In a detailed histological analysis of demyelination in

SJL mice infected with the DA strain, relatively few oligodendrocytes were present in the lesions,¹⁸ evidence that they are the targets for the virus. Viral antigen is detected by immunochemical staining in such cells and complete virus particles have been observed in oligodendrocytes at the inner edge of a demyelinating lesion next to normal white matter (Figure 1a,b).¹⁹

In considering the evolution of chronic disease in mice infected with Theiler's virus, two important questions need to be addressed: where and in what form does the virus persist in the host? and how does virus trigger the disease process? Not surprisingly oligodendrocytes have been identified as sites for virus persistence, based on the detection of virus in the spinal cord of chronically-infected mice by *in situ* hybridisation and immunochemical staining.²⁰ Two staining patterns emerged: in one, 90% of infected glial cells contained 100-500 copies of the viral genome but no viral antigen, taken as evidence of persistent virus. In the remaining 10% of glial cells, 1500 genome copies were present with clear evidence of viral capsids. Of the total infected cells, 25-40% were oligodendrocytes, 5-10% astrocytes and 10% macrophage-like.²¹ Theiler's virus has been shown to persist in macrophages *in vitro*,²² thus implicating these cells as carriers of virus in the persistently-infected animals and as initiators of disease in the CNS. Consistent with this hypothesis is the anatomy of the demyelinating lesion, which appears to evolve from the pial surface, thus favouring virus entry from the CSF, probably in mononuclear cells (Figure 2a,b).¹⁹ This Trojan-horse role of the macrophage is a common means whereby other viruses enter the nervous system to cause disease,²³ and see other articles in this issue.

The mechanism whereby this virus persists in the host is still unknown. Analysis of L cell lines persistently infected with the virus indicates RNA replication is blocked at the level of minus RNA synthesis. Interferon may be important in the initiation and maintenance of this process.²⁵ Another mechanism of persistence is the generation of antigenic variants. The fact that antibody escape mutants are relatively easy to generate *in vitro* suggests that they might occur *in vivo*. Consequently, virus might be maintained in the nervous system at undetectable levels by humoral immunity. Due to the oligoclonal nature of the antibody response to this virus in the CNS, antibody escape variants may arise and initiate infection. Another mechanism involving escape from virus neutralisation is the

release of proteases by macrophages, which destroy the major antibody binding site on VP1,²⁶ leading to a possible change in tropism and persistence.

The host response

We can view the host response to Theiler's virus at two levels: genetic resistance (natural immunity) and the role of the immune response. As mentioned earlier, certain strains of mice are susceptible/resistant to demyelinating disease induced by the To, BeAn and DA strains of the virus. Of these SJL mice, and others on the H-2^s background, are particularly susceptible. Susceptibility to Theiler's virus induced demyelinating disease involves both major histocompatibility complex (MHC) and non-MHC genes. The location of two non-MHC linked genes have been identified, one on chromosome 6 located in or near those encoding the β chain of the T cell receptor²⁷ and another on chromosome 3 near the carbonic anhydrase-2(car-2) enzyme locus.²⁸ The region in the MHC associated with susceptibility maps to the class I locus H-2D.^{29,30} Using congenic mice on the H-2^d background, which are normally resistant to virus-induced disease, a mutant-*dm-1* has been identified which has a deletion 3' of H-2D and 5' of H-2L, producing a hybrid class I gene product; this is now susceptible to demyelinating disease.²⁹ In this instance the H-2D locus probably plays an important role in the evolution of disease since the non-MHC background is that of the resistant genotype.

The most likely role for the H-2D gene products is the presentation of viral antigens to MHC class I restricted, CD8⁺ cytotoxic T cells. If this is the case; how is susceptibility to chronic infection linked to this response? At least two possibilities exist: in susceptible mouse strains CD8⁺ T cells fail to recognise viral antigens in the context of MHC class I and so the virus persists and eventually causes disease; and the cytotoxic T cells recognise antigen presented by H-2D and initiate damage by destroying the infected cell. If this cell is a persistently-infected oligodendrocyte, then demyelinating disease could arise by this mechanism.³¹ We recently explored the role of CD8⁺ T cells in the immune response to Theiler's virus infection of SJL and CBA mice.³² The T cells were depleted *in vivo* by injection of specific cytotoxic monoclonal antibodies. The results show that virus clearance during the acute infection is delayed and there is an increase in the number of

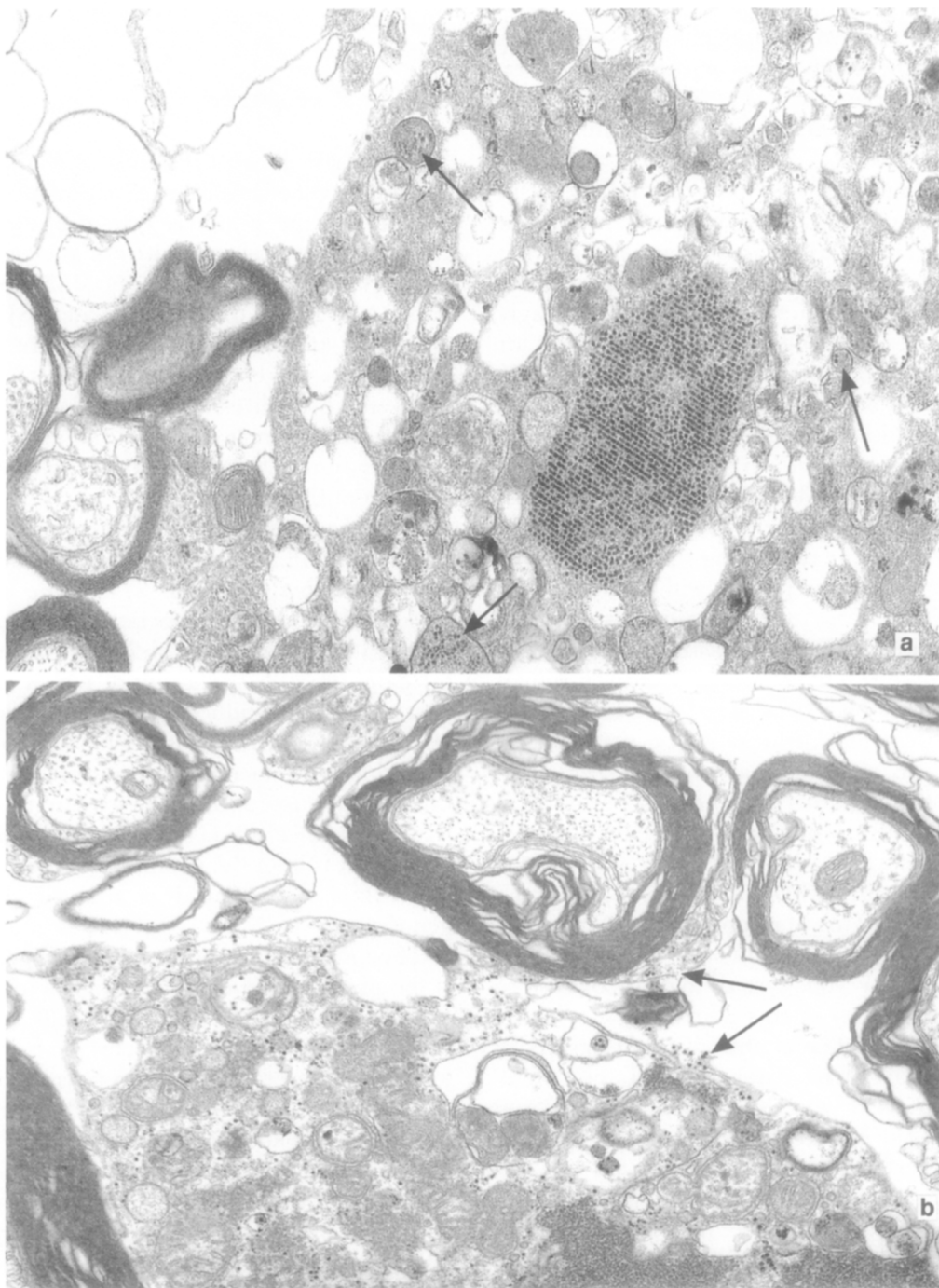


Figure 1. Theiler's virus particles in oligodendrocytes located at the inner edge of a lesion, next to normal white matter. (a) A paracrystalline array of virus particles in a cell with disorganised cytoplasm and margination of nuclear chromatin. Virus particles are seen in some of the membrane bounded structures (arrows). $\times 29,600$. (b) Virus particles in the perinuclear cytoplasm, the extracellular space and outer oligodendrocyte tongues of adjacent myelin sheaths (arrows). $\times 19,200$.

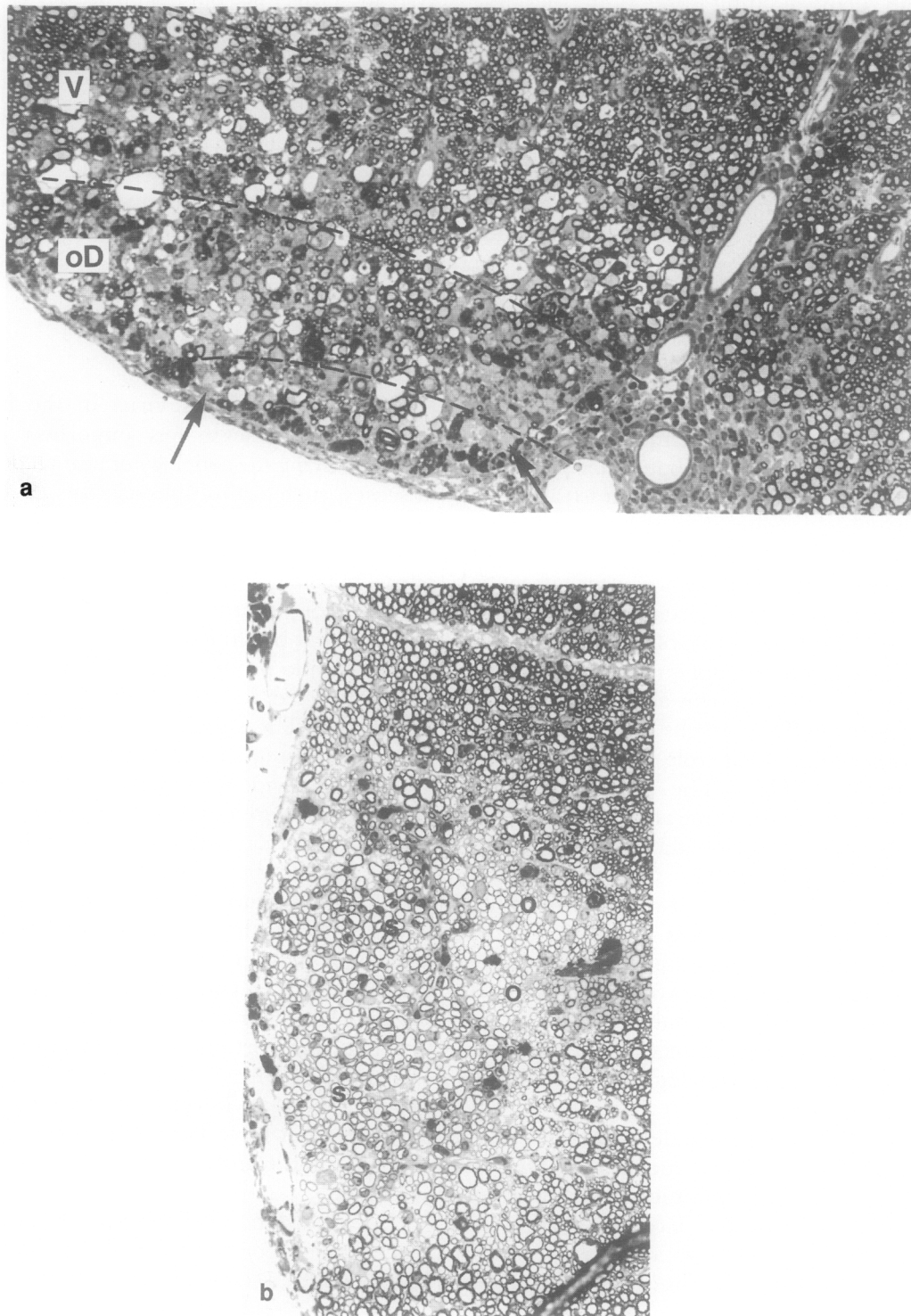


Figure 2. Early and late (repaired) demyelinating lesions in the spinal cord of CBA mice infected with 10^4 pfu of strain BeAn. (a) Active demyelination 130 days after infection. The lines demarcate zones where some demyelinated axons are present (arrowed) next to the pial surface, an area of ongoing demyelination (oD), and a region where virus infected cells are to be observed (V). $\times 480$. (b) A repaired lesion 212 days after infection. Some axons are repaired by Schwann cells (s), others are remyelinated by oligodendrocytes (o). $\times 360$.

animals with demyelinating disease in the first 7 weeks after infection, compared with normal (non-T cell depleted) littermates. These data favour a role for CD8⁺ T cells in antiviral immunity in the acute infection and immune surveillance of persistently infected cells but not in immunopathology. It is also clear from these experiments that CD8⁺ T cells are not vital for recovery from the acute infection. CD4⁺ T cells, on the other hand, are essential for controlling this stage of the infection.

In contrast to the action of CD8⁺ T cells, *in vivo* depletion of CD4⁺ T cells from CBA or SJL mice infected with BeAn results in an overwhelming infection of the CNS, with animals dying by the fourth week.¹⁶ The depleted mice make no antibody, in contrast to untreated controls, but can be protected from a lethal infection by the administration of neutralising antibody against the virus. This supports many studies which highlight the importance of antibody in the recovery from picornavirus infections (e.g. coxsackie virus B3, poliovirus, foot-and-mouth disease virus).³³ The major role of CD4⁺ T cells in this type of infection is probably to provide help for B lymphocytes. Little is known about the possible antiviral activity of CD4⁺ T cells in Theiler's virus infection, other than their role in delayed type hypersensitivity (DTH). The DTH response increases in magnitude with time after infection. The peak response occurs during the chronic disease phase, when the animals are showing signs of demyelination. This parallel between the intensity of DTH and clinical signs was noted by Clatch *et al*,³⁴ who argued that this immunological response was a contributing factor to demyelinating disease. They observed that DTH was only apparent in the disease-susceptible mouse strains. This raises an important issue about the pathological role of CD4⁺ T cells in this infection.

There is now clear evidence demonstrating that suppression of CD4⁺ T cell function *in vivo* by either using anti-Ia blocking antibodies,³⁴ or using anti-CD4 antibodies to deplete T cells just before the onset of clinical signs¹⁶ leads to a reduction in the incidence of demyelinating disease. So how do CD4⁺ T cells initiate this pathological response? There are various possibilities: First, these T cells can produce direct damage to virus-infected glial cells. Astrocytes can express MHC class II antigens and present virus to CD4⁺ T cells. Interestingly, *in vitro* studies show that MHC class II antigens are readily induced by γ -interferon on astrocytes from

SJL and CBA mice but not on astrocytes from Balb/c mice (resistant to disease).³⁶ Second, a function of DTH T cells is the ability to induce mononuclear cell infiltration and activate macrophages, which in turn can lead to damage of myelinated nerves—the so called bystander effect. Third, T cells reactive against nerve cell antigens could be induced during the course of the infection, which may serve to exacerbate on-going pathology or to initiate new lesions.

Autoimmune disease

The role of Theiler's virus in the induction of autoimmune disease has important implications when studying the aetiology of human demyelinating diseases, such as multiple sclerosis. Evidence exists for the induction of autoantibodies and autoreactive T cells against nervous tissue antigens in Theiler's virus infected animals. Antibodies to myelin^{17,37} and proteolipid protein³⁸ (a cross-reactive antibody was isolated binding to VP1 of DA virus and also to proteolipid protein), and DTH to myelin³⁶ have been detected in chronically infected CBA and SJL mice. The significance of these responses to initiating subsequent lesions has not, however, been delineated. Evidence against a role for autoimmune T cells in the induction of this disease process comes from the observation that tolerance induced to myelin basic protein in mice blocked the induction of experimental autoimmune encephalitis (EAE), but did not affect the precipitation of demyelinating lesions in Theiler's virus infected animals.³⁹ This supports earlier conclusions that the virus is the principal initiator of demyelination. In spite of these findings it is worth noting that in rats infected with JHM-strain virus⁴⁰ (a coronavirus) or measles virus,⁴¹ CD4⁺ T cells reactive against myelin basic protein have been identified. Such reactive T cells could transfer an EAE-like disease to naive animals.

In conclusion, Theiler's virus has proved to be a useful model for analysing the pathological events involved in a demyelinating disease. The virus presents several intriguing questions related to persistence, evasion of immune responses and the induction of autoimmune disease. Over the next few years, the application of molecular virology and immunology techniques should go some way to resolving the mysteries surrounding this fascinating neurotropic picornavirus.

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