ANIMAL MODEL OF HUMAN DISEASE

Multiple Sclerosis

Animal Model: Theiler's Virus Infection in Mice

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Biologic Features

Theiler's encephalomyelitis viruses (TMEV) are naturally occurring murine picornaviruses that are a prevalent cause of asymptomatic enteric infection in young adult mice.[1,2] On occasion, viremia with spread to the central nervous system (CNS) probably occurs; there are a number of reported instances of spontaneous flaccid paralysis due to TMEV. Yet, the incidence of spontaneous paralysis is low and has been estimated to be 1 case/5,000 to 10,000 colony-reared mice.[3] Experimental transmission of TMEV by intracerebral and intranasal inoculation produces a similar paralytic disease in mice with necrosis of motor neurons and microglial inflammation as the predominate histopathologic changes in the spinal cord, brainstem, and thalamus.[4,5] While Theiler originally showed that mice develop chronic CNS infection,[4] Daniels and co-workers were the first to recognize that persistent infection resulted in late pathologic involvement of the CNS.[6]

A biphasic disease of the CNS occurs in mice following experimental infection.[7] The early phase of infection is characterized by virus growth, pathologic involvement of gray matter, and flaccid paralysis. We have referred to this phase as early disease. More important, surviving mice develop persistent infection of the CNS with marked mononuclear cell infiltrates in leptomeninges and white matter and demyelination. Demyelination occurs at the same time as a striking gait disorder characterized by spasticity of limbs and stimulus-sensitive extensor spasms. We have referred to this as *late disease*. Infectious virus at low levels is readily detectable in the CNS of most mice for as long as 1 year, in spite of the presence of substantial titers of nutralizing antibody in the serum [8] and CNS.[9]

Pathologic Features

During the chronic stage of infection the lesions are strictly demyelinating. The demyelinating stage of TMEV infection primarily affects the

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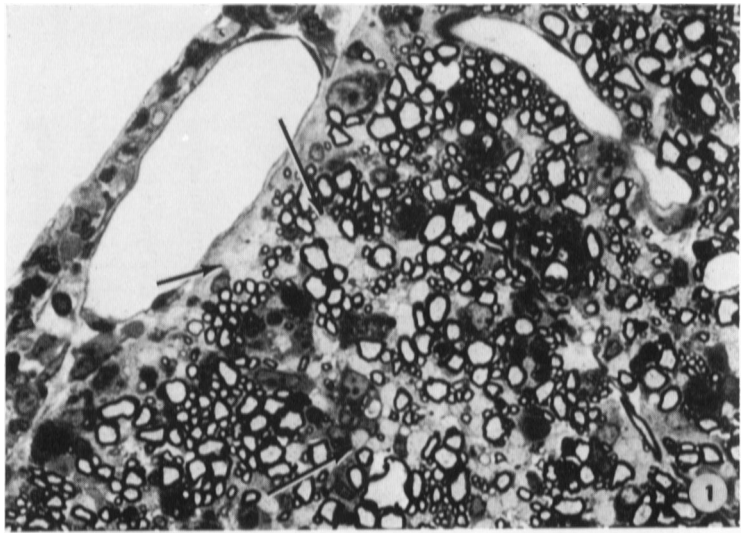
spinal cord.[10] Extensive but patchy areas of leptomeningeal and white matter mononuclear cell infiltrates with concomitant demyelination are present by the third week (Figure 1), reach maximum severity by about 3 months, and are still observed as late as 1 year. The inflammatory infiltrates are composed of lymphocytes, monocytes, and some plasma cells; macrophages containing myelin debris are readily found in areas of myelin breakdown. Remyelinating axons and astrocytic gliosis are most conspicuous at later times after infection. Ultrastructurally, stripping of myelin lamellae by invading mononuclear cell processes (Figure 2) and vesicular disruption of myelin (Figure 3) are the morphologic events accompanying myelin breakdown. Normal oligodendrocytes, the myelin-maintaining cells, are present in the vicinity of demyelinating lesions, and degenerating oligodendrocytes have not been observed. While virus particles have not been found in any CNS cells, this is not unexpected considering the relatively low virus titers and small size of picornavirus virions.

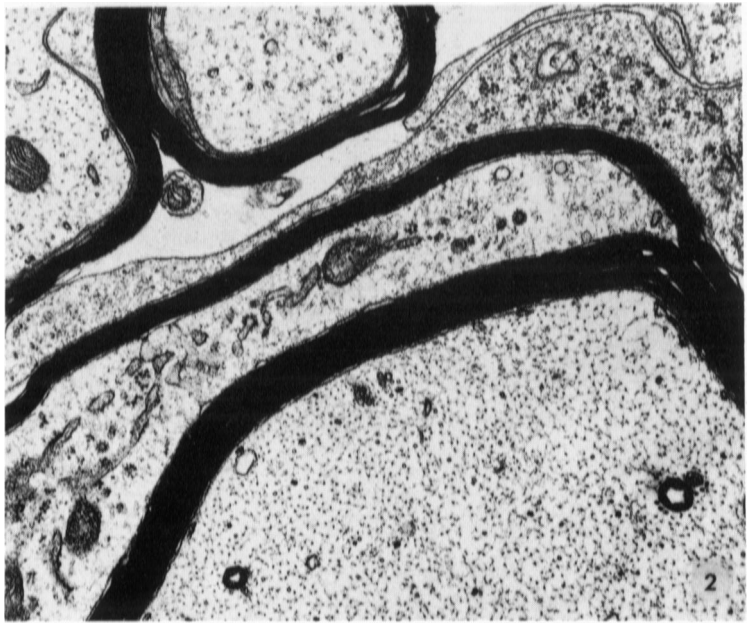
Immunosuppression of this experimental infection with either cyclophosphamide or rabbit antiserum to mouse thymocytes not only eliminates mononuclear cell infiltrates in the spinal cord white matter but also prevents the occurrence of demyelination,[11] suggesting that demyelination in this infection is immune-mediated.

Comparison With Multiple Sclerosis

Multiple sclerosis is a chronic disease of the CNS with manifold clinical symptoms and signs and a distinctive relapsing course. The lesions, essentially limited to white matter are characterized by well-demarcated areas of myelin destruction (plaques) with relative preservation of axons, i.e., primary demyelination.[12] However, the center of larger plaques may be necrotic. Although the earliest lesion in multiple sclerosis remains the subject of controversy, it is generally accepted that acute plaques are associated with cuffing of vessels with mononuclear inflammatory cells. These cuffs are composed of small lymphocytes, some plasma cells, and macrophages.

It has been suggested that demyelination in multiple sclerosis may result from an immune-mediated response triggered by a virus infection of the CNS. Nonetheless, evidence that a virus causes multiple sclerosis is still circumstantial. However, one compelling reason for seriously considering a viral cause is the existence of experimental animal models of virus-induced demyelination, recently summarized by Weiner *et al.*[13] While TMEV infection in mice was not discussed, we feel it is a closer analog of multiple sclerosis than other animal models for the following reasons: a) demyelination is the sole structural change during the chronic phase of





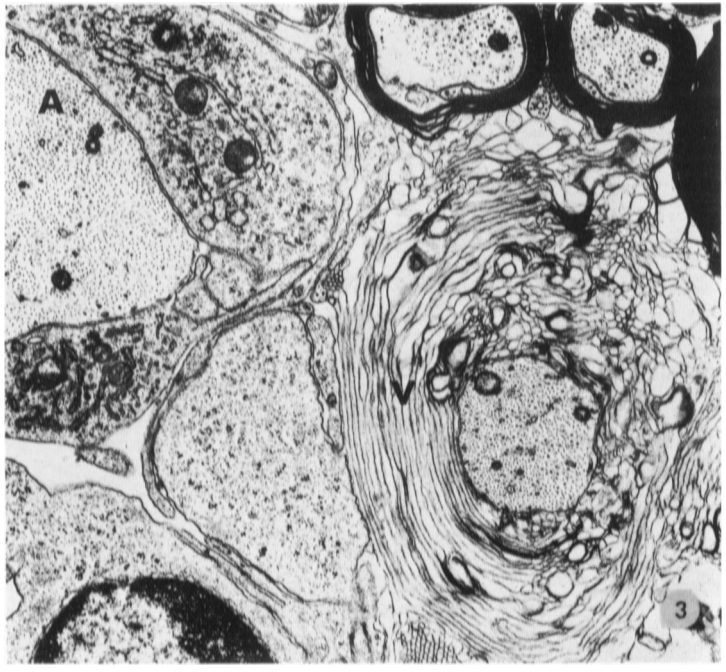


Figure 1—Spinal cord 17 days after intracerebral inoculation of the DA strain of TMEV; Note the leptomeningeal perivascular inflammation on the left, numerous naked axons (*arrows*), and debris-laden macrophages (1-μ Epon-embedded section stained with toluidine blue, × 500). **Figure 2**—Electron micrograph showing a process of a mononuclear cell stripping lamellae from the myelin sheath of a normal-appearing axon (× 5000). **Figure 3**—Electron micrograph illustrating vesicular disruption (*V*) of myelin from an otherwise normal axon; a demyelinated axon (*A*) which is surrounded by mononuclear cell processes can be seen (× 23,000).

infection and neurologic disease in the mouse can be directly attributed to this lesion; b) some mice only develop late disease (the demyelinating stage of this infection) and this occurs after a prolonged incubation period of several months; and c) of greater significance, myelin breakdown appears to be immune-mediated. It should be pointed out that TMEV-infected mice have severe leptomeningitis and the demyelination is essentially limited to the spinal cord. In multiple sclerosis, inflammation is uncommonly seen in the leptomeninges and patchy areas of demyelination are distributed throughout the CNS. The relative paucity of myelin in the cerebral hemispheres of rodents in comparison with those of primates may be responsible for this difference in lesion distribution.

Usefulness and Availability of the Model

TMEV infection in mice represents a practical model system for studying the pathogenesis of virus-induced demyelination. Both DA and Yale strains of TMEV produce demyelinating disease in mice; other TO (Theiler's-original) strains probably will produce similar disease. The Yale strain can be purchased commercially (American Type Culture Collection, Rockville, Md.) and the DA strain can be obtained from the author's laboratory. Inbred SJL/J mice appear to be the optimal host, and they can be purchased commercially (Jackson Laboratory, Bar Harbor, Me.).

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