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Theiler's Virus Infection: a Model for Multiple Sclerosis

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INTRODUCTION

Theiler's murine encephalomyelitis virus (TMEV or Theiler's virus) was first reported by Theiler in 1937 [461] and is a single-stranded RNA virus that belongs to the *Picornaviridae* family. TMEV is responsible for causing neurological and enteric diseases in susceptible strains of mice, such as SJL (reviewed by Dal Canto et al. [86], Monteyne et al. [299], Oleszak et al. [328], Tsunoda and Fujinami [475], and Lipton and Jelachich [250]).

TMEV is a member of the *Cardiovirus* genus. Its genome consists of single-stranded RNA of positive polarity (322, 341, 354) comprising approximately 8,100 nucleotides. The genomic organization of TMEV follows that of standard picornavirus genomic format (L-4-3-4). It codes for 12 proteins arranged in the order 5′-L, VP4, VP2, VP3, VP1, 2A, 2B, 2C, 3A, 3B, 3C, 3D-3′. The 76-amino-acid long L protein is a zinc-binding metalloprotein (74), but its exact function is not fully known. VP4, VP2, VP3, and VP1 are capsid proteins. Proteins 2A, 2B, 2C, 3A, 3B, 3C, and 3D are required, directly or indirectly, for viral RNA replication.

TMEV Subgroups and Different Strains of the Virus

Two major subgroups of TMEV have been reported, and they are distinguished primarily on the basis of their different neurovirulence, antigenicity, and other characteristics (86, 87, 234, 248). The first subgroup includes the GDVII and FA strains, which are extremely neurovirulent variants that induce only acute encephalitis and do not persist in the very few animals that survive the infection. The second subgroup is known as Theiler's original (TO) and includes the BeAn and DA strains. Members of the two subgroups, particularly in the GDVII, BeAn, and DA strains, have been sequenced and extensively characterized (127, 128, 246). Although the capsid proteins of the BeAn and DA strains have 93% amino acid homology, it is well established that the disease induced by the BeAn strain in SJL mice is different from the disease induced by the DA strain. Early acute disease (see below) is more attenuated in BeAn-infected mice in comparison to the distinct grey matter disease induced by the DA strain of TMEV (86, 87). Although both BeAn and DA strains induce late chronic demyelinating disease (see below), the kinetics of the disease caused by the two strains is also different. BeAn-infected SJL mice develop clear clinical signs, such as waddling gait and hind leg paralysis 30 to 40 days postinfection (p.i.) or 50 days p.i. (175), depending on the dose of the virus and the age of the animals. In contrast, DA-infected SJL mice develop such signs much later, at approximately 140 to 180 days p.i. The differences and similarities in the neurological disease induced by the DA and the BeAn strains of TMEV in SJL mice are summarized in Table 1.

TMEV Infection: Early Acute Disease and Late Chronic Demyelinating Disease

Intracranial (i.c.) inoculation of susceptible strains of mice with the DA strain induces biphasic disease, consisting of early acute disease, which occurs within 3 to 12 days p.i., followed by late chronic demyelinating disease, which develops at 30 to 40 days p.i. and eventually causes the death of the animals (reviewed by Dal Canto et al. [86, 88], Monteyne et al. [299], Oleszak et al. [328], Theiler [461], and Tsunoda and Fujinami [475]). In contrast, resistant strains of mice, such as C57BL/6 (B6), develop only early acute disease, clear the virus completely in about 3 weeks p.i., and do not develop late chronic demyelinating disease (245, 260).

Early acute disease resembles polioencephalomyelitis and is associated with replication of the virus in the central nervous system (CNS) gray matter (10^6 to 10^7 PFU/g of CNS tissue) and with destruction of neurons to a variable degree. During acute early disease, both susceptible and resistant strains of mice exhibit extensive mononuclear cell infiltrates in the CNS, consisting of T cells (of both CD4 and CD8 phenotypes), cells of the monocyte/macrophage lineage, few B lymphocytes, and few plasma cells (reviewed by Rodriguez et al. [384], Oleszak et al. [328], Begolka et al. [28], Drescher et al. [109], Murray et al. [308], and Pope et al. [356]). Early acute disease is not always clinically apparent (245, 260, 447, 461), and the severity of this phase of TMEV pathogenesis depends on the strain and the dose of the virus. Viral titers in TMEV-infected susceptible mice are greatly reduced by 12 days p.i. However, TMEV-infected susceptible strains of mice fail to completely clear the virus, which persists in monocytes/macrophages, microglia, astrocytes, and oligodendrocytes (77, 180, 364, 447, 475).

At 30 to 40 days p.i., TMEV DA-infected susceptible mice develop late chronic demyelinating disease with extensive demyelinating lesions of the white matter and mononuclear cell infiltrates in the spinal cord, consisting primarily of $CD4^+$ and $CD8^+$ T cells, some monocytes/macrophages, and few B cells and plasma cells (reviewed by Rodriguez et al. [384], Oleszak et al. [328], Begolka et al. [28], Drescher et al. [109], Murray et al. [308], and Pope et al. [356]). Late chronic demyelinating

TABLE 1. Comparison of neurological disease induced by DA and BeAn strains of TMEV in SJL mice

Characteristic	DA	BeAn	References
Pathogenesis	Biphasic disease consisting of an early acute disease (polioencephalomyelitis) and late chronic demyelinating disease	Biphasic disease consisting of attenuated grey matter disease followed by late chronic demyelinating disease	250, 386, 474
Early acute disease	Intense and multifocal mononuclear cell infiltrates in the grey matter of brain and spinal cord	Attenuated grey matter disease, clinically inapparent	86, 87, 250, 386, 474
Late chronic demyelinating disease	Clinically apparent at ~140–180 p.i.; demyelinating lesions in the white matter of spinal cords associated with perivascular and parenchymal infiltrates	Clinically apparent at 30–40 days p.i.; demyelinating lesions in the white matter of spinal cords associated with perivascular and parenchymal infiltrates	175, 199, 250, 294, 386, 474
CTL epitopes	*H-2K*^{s} restricted ($VP1_{11–20}$)	*H-2K*^{s} restricted $VP3_{159–166}$, $VP1_{11–20}$ $VP3_{173–181}$	190, 191
$CD4^+$ T-cell epitopes	$VP1_{233–250}$, $VP2_{74–86}$, $VP3_{24–37}$	$VP1_{233–250}$, $VP2_{74–86}$, $VP3_{24–37}$	137, 509, 510
Cytokines	Both Th1 and Th2	Both Th1 and Th2	28, 70, 408, 460
iNOS	Expressed in astroglia and macrophages/microglia; maximal levels during early acute disease, and low levels at early stages of late chronic demyelinating disease (39–42 days p.i.;) not detected at 67 and 180 days p.i.	Expressed in monocytes/macrophages but not in astroglia; low levels at 15 days p.i., maximal levels at 60 days p.i.	175, 334, 396
Apoptosis of inflammatory infiltrates	Predominantly T cells (23–32% of $CD3^+$ cells) and, to a lesser extent, macrophages during early acute disease; minimal during late chronic demyelinating disease (2–5% of $CD3^+$ cells)	Predominantly T cells and macrophages throughout the disease (31–96 days p.i.)	326, 396, 416

disease leads to progressive spinal cord atrophy and axonal loss with ensuing neurological deterioration (disruption in motor coordination, hind limb paralysis, spasticity, ataxia, and incontinence) (109, 206, 207, 250, 283, 284). Low-grade viral replication, at the level of 10^1 to 10^3 PFU/g of CNS tissue, has been well documented in macrophages, oligodendrocytes, and astrocytes in the white matter of the spinal cords of TMEV-infected SJL mice with late chronic demyelinating disease (109, 250, 475). This persistent infection has been observed in these mice for as long as 2 years p.i. (244). In BeAn-infected SJL mice, 20 to 30 copies of viral RNA/μg of total RNA from infected spinal cords were detected at 4 months p.i. by real-time reverse transcription-PCR (472). Few copies of viral RNA could still be detected in infected SJL mice at 1 year p.i. (472). It is not fully understood why the virus persists in the CNS of SJL mice whereas B6 mice are able to clear the infection. Possible mechanisms of viral persistence in SJL mice are discussed later in this review.

The immune responses to the virus of TMEV-infected (DA strain) sensitive (SJL) and resistant (B6) strains of mice are summarized in Fig. 1.

Determinants of Viral Persistence

Viral persistence appears to be a prerequisite to developing late chronic demyelinating disease. However, different mechanisms may be involved in these two processes. We have provided evidence suggesting that persistence of TMEV in the CNS is not sufficient to produce demyelinating disease (331, 345). We have isolated two plaque size variants of the DA strain of TMEV (331). One variant produced small plaques, had a significantly higher growth rate at 37°C, and reached 130- to 500-fold higher titer at 37 than at 39°C. The other variant produced large plaques and had a lower growth rate. Although both plaque size variants and the DA strain were capable of establishing persistent infections, only the small-plaque variant and the DA strain were able to induce demyelinating disease in SJL mice (331). We have also reported persistent infection with the DA strain (10^6 to 10^8 PFU/ml) in the G26-20 glioma cell line in vitro (345). A TMEV variant isolated from the G26-20 glioma cells produced smaller plaques in vitro than did the wild-type DA TMEV strain. i.c. infection of SJL mice with this TMEV variant did not result in viral persistence or in late chronic demyelinating disease. However, total-body irradiation (300 rads) of SJL mice infected with this TMEV variant resulted in viral persistence without the development of late chronic demyelinating disease (345). These results demonstrate that different mechanisms may lead to the development of viral persistence and demyelinating disease.

Many studies have been carried out to identify the molecular determinants of persistence. Chimeric viruses between GDVII (an extremely neurovirulent variant which induces only acute encephalitis and does not persist) and DA or BeAn (which cause persistence and demyelination) strains have been constructed. The major finding of these studies is that the viral capsid plays a major role in persistence (3, 178, 280, 454). Within the capsid, several amino acids have been identified as being involved in establishing persistent infection (178, 409, 426, 524). It has been suggested that persistence depends on a conformational determinant within the capsid requiring homologous sequences in both the VP2 puff and VP1 loop, which

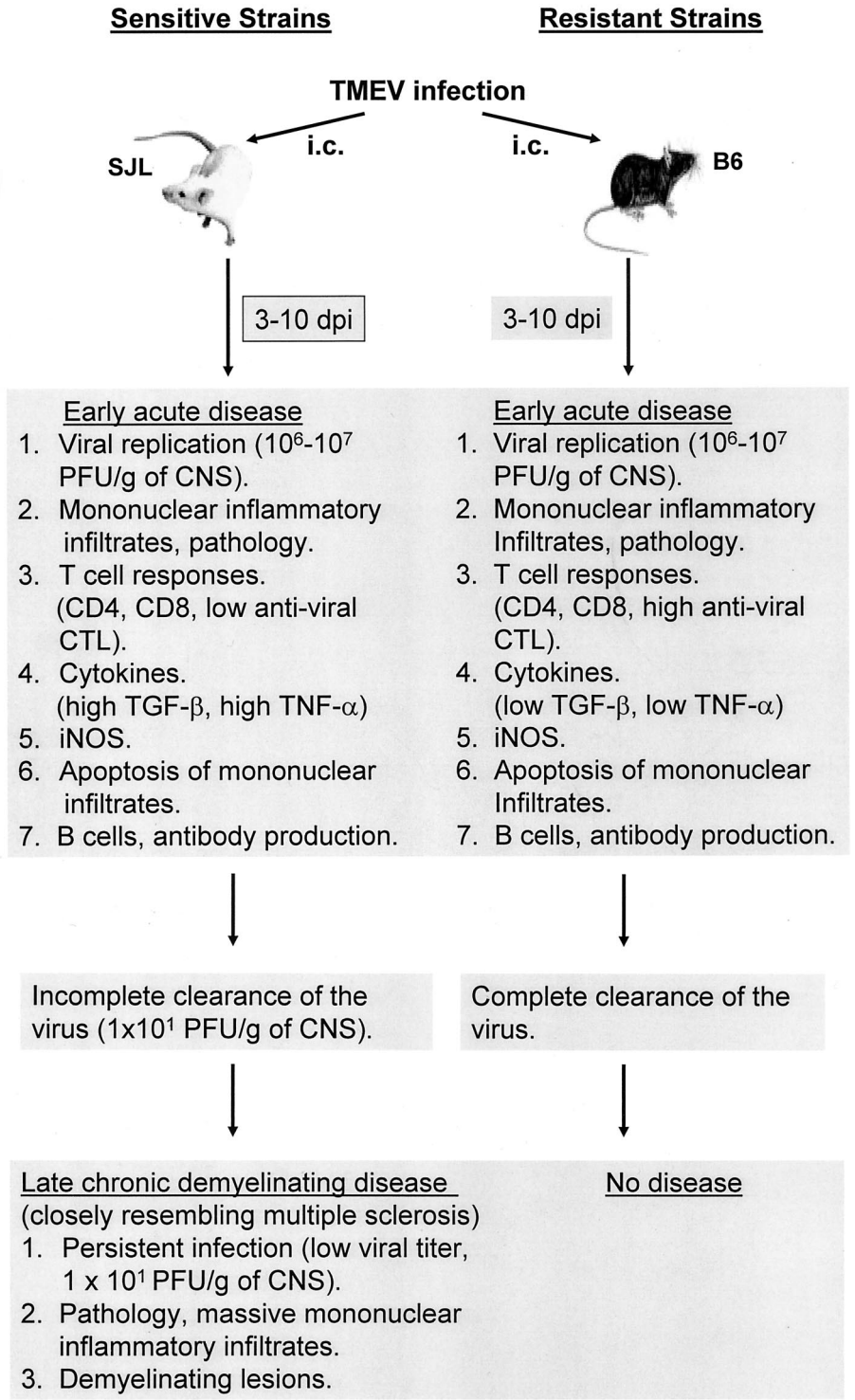

FIG. 1. Comparison of the immune responses to TMEV of sensitive (SJL) and resistant (B6) strains of mice infected i.c. with the virus. Differences in the immune responses of these strains of mice to TMEV are summarized during early acute disease (polioencephalomyelitis). Resistant strains of mice completely clear the virus and do not develop demyelinating disease. In contrast, sensitive strains of mice fail to completely clear the virus and develop persistent viral infection associated with low virus titers and late chronic demyelinating disease, characterized by massive mononuclear cell inflammatory infiltrates and demyelinating lesions.

are in close contact on the virion surface (3, 252, 477). These residues, located around the edge of the "pit," may be in close proximity to a putative receptor-binding site (47). However, conflicting results have been obtained about whether these determinants for persistence and demyelination can be contributed only by the DA and BeAn strains (60, 127, 280, 392) or whether these determinants are also present within the capsid of GDVII virus (3, 127, 392). In general, a chimeric GDVII virus, whose capsid had been replaced by that of the DA or the BeAn strain, was attenuated whereas a recombinant DA or BeAn virus with a GDVII capsid was virulent. However, mere attenuation of the neurovirulence of GDVII is not sufficient to established persistence (179, 252). The significance of conformational differences via interaction of VP2 puff B and VP1 loop II between GDVII and DA viruses has been recently illustrated by generation of specific mutants (477). Thus, the DA virus mutant with a puff B similar to that of GDVII induced both acute and late chronic demyelinating disease similar to that induced by wild-type DA, while the DapBL2M virus with VP1 loop II of GDVII and an additional mutation in VP2 puff B caused only prolonged gray matter disease without demyelination (477). Whether mutations within VP2 puff B and VP1 of the capsid modulate tropism of the virus by altering the affinity of the capsid for the receptor on different cells is not fully determined (186).

The major gene controlling viral persistence is the *H-2D* gene (55, 379, 386). Additional loci controlling viral persistence have been identified, and they are located close to the Ifng locus (in the telomeric region of chromosome 10). They include two loci designated *Tmevp2* and *Tmevp3* (37, 56). A third locus close to the locuse encoding myelin basic protein (MBP) (in the telomeric region of chromosome 18) has also been found (37, 56). Although *Tmevp2* and *Tmevp3* are found close to the Ifng locus, genetic analysis revealed that the IFN-γ gene was excluded from the chromosomal regions containing the *Tmevp2* and *Tmevp3* loci (37). Additional studies have shown that the difference in the Th1-Th2 cytokine balance between TMEV-susceptible SJL mice and two lines of resistant congenic mice is not due to the Ifng locus. Therefore, the Ifng locus does not seem to be responsible for the differences in susceptibility between SJL mice and the resistant congenic mice (298).

TMEV-INDUCED DEMYELINATING DISEASE AND MULTIPLE SCLEROSIS

As discussed above, i.c. infection of susceptible strains of mice with TMEV results in biphasic disease of the CNS, consisting of early acute disease and late chronic demyelinating disease that appears at 30 to 40 days p.i. This late chronic demyelinating disease in the CNS of TMEV-infected susceptible strains of mice is one of the best, if not the best, experimental animal models of multiple sclerosis (MS). Close similarities between MS in humans and TMEV-induced late chronic demyelinating disease in susceptible strains of mice are discussed in the following section.

Susceptibility of Mice to TMEV-Induced Demyelinating Disease and of Humans to MS is MHC Dependent

Susceptibility of mice to TMEV-induced demyelinating disease is associated with the *H-2D* class I haplotypes s, q, r, p, and f (14, 22, 76, 78, 121, 251, 345, 361, 379, 380, 383, 385, 390). Susceptibility of humans to MS is associated with the DRBI*1501, DQA1*0102, and DQB1*0602 molecular haplotypes and either the DPB1*0401 or DPB1*0402 molecular haplotypes. These molecular haplotypes correspond to the cellular types DR2 and Dw2 (441, 444, 457, 485). Approximately 57% of Caucasian patients with MS are $DR2^+/Dw2^+$ whereas this cellular type is expressed in only 30% of the normal Caucasian population. The remaining patients with MS are $DR2^-$ $Dw2^-$ and represent a patient population that has not been sufficiently studied.

Comparative Neuropathology of TMEV-Induced Demyelinating Disease in Mice and MS in Humans

Neuropathology of TMEV-induced disease. As discussed above, TMEV infection of SJL mice results in early acute disease and late chronic demyelinating disease of the CNS. Early acute disease is characterized by variably intense and multifocal inflammation involving cerebral and spinal cord gray matter (247, 250, 386, 475). The inflammatory infiltrates are mononuclear and consist of lymphocytes (predominantly T cells) and monocytes/macrophages. Although there is lymphocytic infiltration of the leptomeninges and the cerebral cortex, the bulk of the inflammation is concentrated in the subcortical gray matter, especially in the regions of the diencephalon (thalamus, hypothalamus, and subthalamus), the hippocampus (stratum pyramidale), and the basal ganglia (pallidum and caudoputamen). In the spinal cord, the inflammation is concentrated predominantly in the anterior horns of the gray matter, although infiltration of the leptomeninges is also present. During early acute disease, the white matter is unaffected throughout the neuraxis.

The inflammatory mononuclear infiltrates have a distinctive perivascular predilection, in that they often traverse the walls of small and medium-sized parenchymal blood vessels, leading to vasculitis (243). Besides dense perivascular cuffing, there is evidence of sparse inflammatory infiltration in the nearby gray matter neuropil. A significant number of perivascular T lymphocytes exhibit apoptotic features (326). Foci of ischemic-type coagulative necrosis are detected in the diencephalon and hippocampus near dense perivascular inflammation, suggesting vasculitis (C. D. Katsetos, C. D. Platsoucas, and E. L. Oleszak, unpublished data).

As early as 30 days p.i., mononuclear inflammatory infiltrates of the spinal white matter consisting predominantly of T cells and monocytes/macrophages have been documented; they coincide with the onset of late chronic demyelinating disease. Histologically, demyelination is characterized by vacuolar change of the white matter, overt myelin loss, and appearance of myelin-laden phagocytic macrophages within the lesions. Axonal swellings (spheroids) are detected within demyelinating lesions during the advanced stages of chronic disease (283, 284). The demyelinating process is multifocal and involves anterior, posterior, and lateral columns. Although there is an

TABLE 2. Aspects of comparative neuropathology

Characteristic	TMEV infection	MS
Temporospatial gradient	Biphasic	Polyphasic (common), monophasic (less common)
	Early acute disease (~2 wk p.i.) with polioencephalomyelitis (gray matter inflammation)	
	Late chronic demyelinating disease (begins ~35 days p.i.), with demyelinative myelopathy (inflammatory demyelination involving the spinal cord)	Predominantly disseminated white matter lesions throughout the CNS; unusual cortical and deep gray matter involvement
Topography	Early acute disease	
	Gray matter lesions (thalamus, hypothalamus, hippocampus, basal ganglia, mesencephalon, cerebral cortex, spinal gray matter [anterior horns])	
	Late chronic demyelinating disease	
	White matter lesions (spinal cord [all funiculi, but particularly posterior and lateral columns])	Lesions of the periventricular white matter, convolutional white matter, optic tracts, cerebellar peduncles, brain stem (white matter tracts), and spinal cord white matter
Morphologic features		
Inflammatory infiltrates[a]	Present (early and chronic phases)	Present (early active and chronic inactive lesions)
Perivascular inflammation[b]	Present (common)	Present (common)
Gray matter inflammation	Present (early phase only)	Uncommon
Disruption of the blood-brain barrier	Present (early onset)	Present (early onset, throughout the CNS)
Demyelination	Present (chronic phase; predilection for spinal cord)	Present (early onset, throughout the CNS)
Oligodendroglial damage[c]	Present	Present
Remyeliation	Present	Present (usually abortive)
Axonal damage[d]	Present	Present

[a] Inflammatory infiltrates in both TMEV infection and MS consist predominantly of lymphocytes (T and B cells) and cells of the monocyte/macrophage lineage (blood-borne monocytes/macrophages) and activated/transformed resident CNS microglia. Plasma cells are present but to a lesser degree, particularly in MS.

[b] Perivascular lymphocytic-monocytic infiltrates are hallmark features in both TMEV infection and MS. They involve small and medium-size blood vessels, usually postcapillary venules and veins. Inflammation through the blood vessel wall is frequently observed, which suggests an element of vasculitis.

[c] Demyelinating lesions in TMEV infection and MS have similar morphological patterns of oligodendrocyte damage and cell death, including the distal or dying-back oligodendrogliopathy pattern.

[d] TMEV infection and MS share features of axonal damage, which are strong correlates of neurological disability. To date, the pathogenesis and temporospatial profile of axonal injury are not fully elucidated in either disease. In TMEV myelopathy, axonal loss closely relates to spinal cord atrophy incurred in the late stage of chronic disease (100 to 200 days p.i.), that is, after demyelination has reached a plateau.

apparent preferential susceptibility of the thoracic segments, demyelination is present throughout the rostrocaudal length of the spinal cord (386). The demyelinating lesions are accompanied by predominantly perivascular infiltrates consisting of lymphocytes and monocytes/macrophages (109, 250). There is widespread inflammatory infiltration of the spinal leptomeninges. Perivascular, leptomeningeal, and/or neuropil-infiltrating lymphocytes from the chronic lesions lack apoptotic features, in contrast to the prominent apoptosis of lymphocytes during early acute disease (326).

(i) Inflammatory demyelination. The immunological mechanisms of TMEV-induced demyelination are discussed elsewhere in this review. Briefly, both $CD4^+$ and $CD8^+$ T cells infiltrating the CNS of TMEV-infected mice contribute to demyelination. Autoimmune responses to myelin antigens generated during epitope spreading may also play a role in propagating the disease (see below). Additional immunological, metabolic, and toxic factors (discussed in the section on the pathogenesis of demyelination in human MS), acting on myelin sheaths, myelin-forming oligodendrocytes, and/or the axons themselves, may also be involved in TMEV demyelination (see below). A pattern of oligodendroglial damage, which is similar to that encountered in MS (174, 267), is a form of retrograde ("dying back") degeneration beginning in the most distal cell processes of oligodendrocytes near nerve axons in TMEV-infected mice (378, 386). A comparison of the neuropathological features of DA strain-induced neurological disease in mice (early acute and late chronic demyelinating disease) and human MS is presented in Table 2.

(ii) Remyelination. Variable degrees of remyelination are present within TMEV demyelinating lesions (284, 407). The frequency of medium or large remyelinated axons within a single lesion may be a powerful indicator of axonal preservation (407).

(iii) Axonal damage. In the advanced chronic phase of the demyelinating disease, that is, between 100 and 200 days p.i., there is a marked increase in spinal cord parenchymal atrophy without a concomitant increase in spinal cord demyelination (283). A morphological and ectrophysiologic study by McGavern et al. (283) has shown a statistically significant loss of medium-sized and large myelinated axons only after the demyelinating phase of the disease was established. The authors

(283) speculate that following myelin denudation, the naked axons are vulnerable to further inflammation and ultimately succumb to secondary damage (283). This hypothesis is consistent with our findings that axonal swellings occur next to areas of inflammation in advanced-stage chronic demyelinating lesions (Katsetos et al., unpublished). It is unclear whether the axonal damage in TMEV infection is the result of direct (inflammatory) injury or of delayed Wallerian or dying-back type degeneration, accounting for the delayed development of axonal loss (283). In contrast, Tsunoda and Fujinami (474) have recently advanced the assertion that in TMEV-infected mice, axonal injury accompanied by oligodendrocyte apoptosis precedes demyelination, implying that axonal injury can trigger demyelination. The pathogenesis of axonal damage in experimental demyelinating disease should be the subject of further studies. Improvement of our understanding of the mechanisms of axonal damage may have potential therapeutic implications.

Neuropathology of MS. MS is presently defined as an inflammatory demyelinating disease of the CNS. MS lesions are heterogeneous and characterized by inflammation, demyelination, and variable degrees of axonal damage (228, 466). Historically, the morphological hallmark of the disease is the MS plaque; however, the MS plaque represents an end-point anatomical lesion. Accordingly, MS lesions should be viewed in the context of plaque evolution. MS plaques reflect a continuum of immunological activity encompassing variable degrees of inflammation together with new or residual neural damage and secondary reactive cellular changes in the affected brain tissue. Morphologically, plaques are grouped as acute (active), chronic active, chronic inactive, and "shadow plaque" types (157, 466). The cellular heterogeneity and the age of the plaques may be part of a temporospatial gradient of the same pathological process, or, conversely, it may reflect distinct morphological patterns of divergent immunological mechanisms (157, 228). Moreover, it should be emphasized that although the inciting pathological process may be fundamentally inflammatory, consistent with immune-mediated responses, the clinically defined neuropathological lesions seem complex and variegated, involving a combination of immunological, metabolic, and/or other neurotoxic pathways (227, 228).

(i) Acute (fresh) lesions. Acute lesions are the precursor lesions of the MS plaques. They are typified by perivascular infiltration of inflammatory cells (predominantly T lymphocytes and monocytes/macrophages), edema, myelin swelling, and activation of endothelial cells (157, 228, 360, 367, 466). Variable although often pronounced depletion of oligodendrocytes is present (340, 358, 359, 466). Plasma cells are infrequent. In general, there is preservation of axons, but variable degree of axonal damage may be present (466, 467).

(ii) Chronic active lesions. Chronic active lesions are by definition older lesions (plaques) exhibiting areas of active inflammation and demyelination, typically at their margins (the interface with normal tissue). They are characterized by perivascular lymphocytic infiltrates, ongoing myelin breakdown, myelin phagocytosis by foamy macrophages, reduction in the number of oligodendrocytes, and reactive astrocytosis. The lymphocytic infiltrates may extend beyond the margin of overt demyelination (157, 360, 367, 466). There is sparing of axons, but variable degrees of axonal damage may also be present (466, 467).

(iii) Chronic inactive lesions. Chronic inactive lesions are older, "burnt-out" or quiescent lesions, tantamount to glial scars. They are sharply delimited from the adjoining normal myelinated tissue. There is astrocytic gliosis, loss of oligodendrocytes, and variable axonal loss (157, 360, 367, 466, 467). Scant perivascular lymphocytes (cuffs), monocytes, and/or plasma cells may be focally persistent. The walls of the blood vessels may be sclerotic and hyalinized, denoting an antecedent inflammatory vascular injury. A thin rim of perivascular collagenous fibrosis may be present in long-standing lesions. There is disruption of the blood-brain barrier (223).

(iv) "Shadow plaques." Shadow plaques consist of variably sized and ill-defined zones of partially demyelinated or incompletely remyelinated tissue surrounding (and occasionally "overshadowing") the principal plaque (368). Their occurrence in chronic MS is unpredictable, ranging from absent to frequent. There are no known clinical correlates, but shadow plaques may underlie a distinctive pathogenetic pathway (157).

Inflammatory demyelinating lesions are typically disseminated throughout the CNS white matter, but are more frequently present in the optic nerves, brain stem, cerebellum (including the cerebellar peduncles), and spinal cord. Distinctive anatomical correlates are encountered in certain variants, such as the neuromyelitis optica or Devic type of MS (see below). As a rule, involvement of these frequently affected sites leads to neurological symptoms and signs. In the cerebral hemispheres, the lesions exhibit predominantly, but not exclusively, a periventricular distribution. Lesions involving convolutional white matter subjacent to the cerebral cortex typically spare subcortical myelinated fibers (U fibers). Occasionally, white matter lesions extend into the contiguous cortex or deep grey nuclei (basal ganglia and thalami) (reviewed by Prineas and McDonald [360]). Cortical involvement by MS lesions has been described as an additional contributor of neurological disability (353). Exceptionally, atypical solitary, mass-like lesions resembling tumors are encountered (202).

The vast majority of MS cases (~80 to 85%) manifest as the classical (Charcot) type, also known as remitting-relapsing and secondary progressive MS. The remitting-relapsing phase of the disease lasts for 10 to 15 years and is frequently followed by a phase of progressive neurological disability referred to as secondary progressive MS (reviewed by Trapp et al. [466]). The plaque evolution, described above, mirrors the protean activity of lesions in classical MS. Although inflammatory demyelination is undoubtedly a major component of the MS lesion, it does not explain the nature of the neurological disability, which seems to be related more to the degree of underlying axonal damage (213, 262, 466) (see below). Balo's concentric sclerosis is an "anatomical subtype" characterized by a distinctive topographic distribution of MS lesions in which bands of demyelinated white matter alternate with ribbons of unaffected white matter in a concentric fashion (82, 157). A minority of MS cases (~10%) exhibit an unremitting, albeit variably severe, course of clinical progression and are designated primary progressive MS (52, 185, 281).

The acute (Marburg) type of MS may be viewed as the fulminant end of the nosological spectrum of primary progressive MS. It is characterized by a rapidly and relentlessly progressive neurological deterioration, often leading to death within 1 year from the onset of the illness (157, 360). Although

most cases arise and progress de novo, acute MS may also be superimposed on instances of remitting-relapsing MS (157). Myelinoclastic diffuse sclerosis (Schilder's disease) has certain similarities to and pathological features in common with the acute (Marburg) type of MS (334, 360) and is another relatively rare form of acute MS.

The neuromyelitis optica (Devic) type of MS is typified by combined involvement of the optic nerve and the spinal cord. The disease is often fulminant, leading to partial or total loss of vision and extensive spinal cord involvement. Besides demyelination, areas of confluent parenchymal necrosis, consistent with spinal cord ischemic infarction, are often present, culminating in atrophy and cavitation (cystic necrosis) of the affected segments of the spinal cord (157, 360). The active lesions are accompanied by inflammatory infiltrates, consisting predominantly of perivascular T lymphocytes and monocytes/macrophages (157). From the standpoint of preferential spinal cord involvement, Devic disease shows a remarkable similarity to the myelopathic pattern encountered in the late chronic demyelinating disease of TMEV infection.

(v) Inflammatory demyelination. Morphologically, the two integral components of MS lesions are perivascular inflammation and demyelination. It has long been hypothesized that inflammatory demyelination is the result of immune-mediated responses to myelin antigens either in the myelin sheaths of axons and/or at the level of myelin-forming oligodendrocytes (reviewed by Prineas and McDonald [360] and Lassmann [228]). Destruction of myelin and oligodendrocytes is not uniform in MS plaques (157, 360). In spite of decades of intensive research, the mechanism(s) of demyelination currently remains unresolved. However, there is clear evidence of early disruption of the blood-brain barrier and infiltration of the brain substance by blood-borne monocytes and T lymphocytes (228, 360, 466). Importantly, blood-brain barrier disruption relates to the onset of clinical symptoms, but a correlation between symptoms and inflammatory demyelination remains circumstantial.

Four fundamentally distinct patterns of demyelination (I to IV) have been proposed on the basis of myelin protein loss, topography and spatial extension of the lesions/plaques, patterns of oligodendrocyte damage, and immunopathological features of complement activation (264).

The current view is that inflammation composed predominantly of lymphocytes and monocytes/macrophages can cause demyelination by direct and/or indirect mechanisms. Lymphocytes contribute to the pathological process through cellular and humoral immunological responses (presumptive direct mechanisms) or by the production of cytokines (indirect mechanisms). Patterns I and II exhibit remarkable similarities to either T-cell-mediated or T-cell-plus antibody-mediated autoimmune encephalomyelitis (264). It is claimed that the other two patterns (III and IV) are consistent with a primary oligodendrocyte pathology (dystrophy) reminiscent of direct virus- or toxin-induced demyelination, as opposed to autoimmune mechanisms (264).

Monocytes/macrophages participate in the demyelinating process in a dual manner. Besides their traditional phagocytic role (ingestion and removal of myelin debris), cells of the monocyte/macrophage lineage, including blood-borne and activated/transformed resident microglia of the CNS, are potent effectors of axonal myelin and oligodendrocyte damage (466). Monocytes contribute to demyelination through the production of cytokines, nitric oxide, and proteases and/or by directly targeting oligodendrocytes at the border of MS lesions (352, 360, 466). Activated CNS resident microglia play a role in the early stages of demyelination through cell-to-cell contact with myelin internodes of the axons at the edges of active and chronic active MS lesions (reviewed by Prineas and McDonald [360] and Trapp et al. [466]).

Collectively, the formation and evolution of MS plaques require the interaction of complex immunological and metabolic factors including the effect of cytotoxic T cells, antibodies, toxic metabolites derived from activated monocytes/macrophages, and metabolic defects of oligodendrocytes (18, 227, 263, 264, 313). The pathogenesis of myelin destruction in MS appears to be complex and heterogeneous and reflects different patterns of demyelination. Furthermore, MS plaques may represent a common morphological end point of divergent immunological pathways involving myelin and axonal damage (263, 265, 348).

(vi) Oligodendroglial damage and abortive remyelination. Over the years, various theories have been advanced concerning the nature of oligodendrocyte damage in MS lesions. The prevailing, albeit circumstantial, view is that this damage is incurred through a variety of immunological mechanisms, including anti-myelin oligodendrocyte glycoprotein (MOG) antibodies, production of proinflammatory cytokines by monocytes/macrophages and lymphocytes, T-cell-mediated injury (through $CD8^+$ class I major histocompatibility complex [MHC]-restricted cytotoxicity), immunoglobulins and components of activated complement, apoptosis, and a variety of other oligodendrogliotoxic factors (136, 227, 263, 313, 466). As mentioned above, patterns III and IV of demyelinating injury in MS are attributed to some form of oligodendroglial dystrophy (264). In pattern IV there is extensive degeneration and death of oligodendrocytes in the white matter around active lesions (264). In pattern III there is preferential loss of myelin proteins in the distal-most (periaxonal) cell processes of oligodendrocytes, which is associated with oligodendrocyte apoptosis (174, 227, 264). This distinctive mechanism has previously been defined as distal or dying-back (oligodenro)gliopathy (267). Lassmann has recently reappraised the latter pattern in the context of MS, suggesting that it resembles the oligodendroglial pathology incurred during early hypoxic-ischemic demyelination of the white matter (227).

In new MS lesions, both oligodendrocytes and myelin are actively destroyed, a process that in many cases ceases within few weeks. Remyelination frequently ensues following recruitment and repopulation of the plaque by oligodendrocytes (358–360). Evidence of oligodendrocyte precursor cells migrating toward demyelinated lesions has recently been found (69). As a rule, new lesions undergo variable degrees of remyelination, which is, however, either interrupted or confounded by recurrent activity (360).

There is clear ultrastructural evidence of attempted, but generally abortive, remyelination, which is particularly well illustrated in the paradigm of shadow plaques (360, 368). Why remyelination is not distributed uniformly within a lesion or why it is not seen in all cases of MS is not known. However, this

adds to the notion that MS may be the clinicopathological end point of divergent pathogenetic mechanisms (157, 263, 340).

(vii) Axonal damage. Axonal damage in the form of axonal swellings and transection was described in the early literature but was underrated or dismissed as merely an epiphenomenon (reviewed by Kornek et al. [213]). In recent years there has been a critical reappraisal of axonal damage in MS, so much so that it has emerged as a major component of the disease (117, 466, 467). Importantly, axonal injury correlates with certain parameters of functional magnetic resonance imaging (MRI) (reduction of *N*-acetylaspartate) and neurological disability in MS (43, 213, 262, 263). Axonal pathology, evidenced by amyloid precursor protein staining, is more prominent in active MS lesions than in chronic inactive plaques (213). Because axonal injury is likely to be irreversible, early neuroprotection is a paramount therapeutic target.

Several unanswered questions are at issue. First, it is unclear whether axonal damage is secondary to or is sustained concomitantly with myelin damage (90), or whether in fact, it occurs independent of demyelination. Bitsch et al. (41) showed that axonal injury in MS, as determined by immunoreactivity for amyloid precursor protein, is partly independent of demyelination but relates to the number of monocytes/macrophages and $CD8^+$ T cells in MS lesions. Second, although it appears that axonal pathology relates, in part, to the activity of the disease, its temporospatial profile needs further elucidation. Third, the pathogenesis of axonal damage is essentially unknown and may not be entirely immune mediated (263). Finally, the highly variable degree of axonal injury among MS patients may be consistent with the divergent pathogenetic mechanisms of MS.

(viii) Vascular pathology and hypoxic-ischemic damage. Another long-standing yet not universally embraced aspect of MS neuropathology is the distinctive angiocentric distribution of demyelinating lesions, particularly around postcapillary venules and veins (12, 148, 157, 268). Recently, Lassmann (227) revisited Putnam's hypothesis (363) of hypoxic-ischemic type injury as a component of MS lesions. Disturbances in oxidative metabolism resembling hypoxia-ischemia may be the consequence of vascular factors and/or the production of toxic metabolites typically associated with hypoxia-ischemia. Inflammatory damage of the vessel wall, endothelium, and blood-brain barrier by T cells and monocytes is tantamount to a vasculitic state, reminiscent of the angiocentric T-cell infiltrates in human immunodeficiency virus type 1-associated CNS disease (197). The latter, compounded by edema and disturbance of the cerebral microcirculation, may culminate in variably pronounced hypoxic-ischemic injury causing damage to myelin, axons, and oligodendrocytes (227).

Whereas overt ischemic damage has been demonstrated in severe cases of acute MS, Balo's concentric sclerosis, and neuromyelitis optica (82, 266, 490), it is thought that most active MS lesions may represent a form of "sublethal" hypoxic injury reminiscent of ischemic white matter damage. Evidence of hypoxia-like metabolic tissue injury in MS due to the liberation of excitotoxins and reactive oxygen species lends further support to this hypothesis (44, 93, 227, 256, 334). Whether hypoxic-ischemic injury is associated with any viral infection(s) of the CNS remains to be determined.

Viral Etiology of MS

Viral etiology and genetic background appear to play a substantial role in the susceptibility of mice to TMEV infection and of humans to MS. Genetic factors play a role in determining susceptibility to MS. It is well documented that about 10% of patients with MS have first- and second-degree relatives with MS (112). As previously discussed, the frequency of certain MHC class II genes is higher in certain patients with MS than in the general population in the same area (441, 444, 457, 485). However, the concordance rate in monozygotic twins is about 25%, clearly indicating that MS is not purely a genetic disease (reviewed in reference 412).

The role of environmental factors in MS has been suggested by numerous studies. Epidemiological studies that examined the migration of populations between low- and high-incidence zones indicate that there is a profound North-South gradient in disease incidence (39). Further, these studies strongly suggest that an infectious agent(s), possibly a virus(es), may be involved and that the infectious agent is likely to be acquired before 13 to 15 years of age (219). In addition, studies of MS "epidemics" occurring in areas with previously low incidence of MS, preceded by the introduction of new infectious agents (such as in the Faroe Islands and Sardinia), strongly argue for the role of an environmental factor(s) in triggering the disease (29, 218, 220–222, 395).

A number of viruses have been isolated from the CNS of patients with MS; however, it is not clear whether they are endogenous viruses of the CNS or pathogens triggering the disease. Paramyxoviruses are particularly interesting since the "epidemic of MS" in the Faroe Islands followed the introduction of canine distemper virus (CDV) into the island by British troops. It has been suggested that dogs infected with CDV may have transmitted the disease to humans. Patients with MS have higher titer of measles virus than does the control population (4). Simian virus-5 (another member of the *Paramyxoviridae* family), which infects both humans and dogs, has also been implicated in MS (119). The list of viruses associated with MS includes coronaviruses, retroviruses, endogenous retroviruses, and several members (Epstein-Barr virus [EBV] and human herpesvirus 6 and 7) of the herpesvirus family (118, 300, 404). Recently, virus-like structures have been isolated from the brains of patients with MS and from the brains of cats (498). However, it appear unlikely that MS is triggered by a single virus. Results obtained with viral experimental models suggest that many different viruses may trigger inflammatory demyelinating diseases resembling MS. Among these viruses are mouse hepatitis virus (a coronavirus), Semliki Forest virus (SFV) (an alphavirus), visna virus (a lentivirus), and CDV (a morbillivirus) (116). However, late chronic demyelinating disease induced by TMEV is an excellent model of MS because of its histopathological and immunological similarities as well as its similar genetic characteristics to MS.

SUSCEPTIBILITY TO TMEV INFECTION IS GENETICALLY CONTROLLED

Evidence has accumulated demonstrating that several loci in the mouse genome are responsible for the genetic control of susceptibility to TMEV infection, viral persistence, and the

development of TMEV-induced late chronic demyelinating disease. The loci involved include the *H-2D* region of MHC class I, the Cβ gene segment of the T-cell receptor (TCR), and a third locus mapped on chromosome 3 (22, 24, 47, 55, 458). Viral persistence is also genetically controlled, as discussed earlier in this review (37, 56). Welsh and coworkers (406, 494, 496) reported that the establishment of persistent infection in the CNS of TMEV-infected mice appears to be associated with the increased expression of *H-2* class I on cerebrovascular endothelial cells. These cerebrovascular endothelial cells may be potentially responsible for presenting antigen to T cells (406, 494, 496). The genetics of TMEV infections has been reviewed elsewhere by ourselves (328, 384) and others (37, 38, 47, 86, 250, 299, 475) and is not discussed further in this review.

TMEV AND MACROPHAGES

Neurons serve as the major targets of infection for the GDVII and DA strains of TMEV during the early phase of TMEV-induced disease (polioencephalomyelitis) (21). However, during late chronic demyelinating disease, macrophages (77, 88, 253, 398) and, to a lesser extent, glial cells (77, 88, 253, 337) serve as the site of TMEV DA persistence. The presence of active viral replication in macrophages is indicated from (i) the immunohistochemical detection of virus associated with cytoskeletal changes in macrophages (88), (ii) the presence of a large TMEV antigen burden (253), and (iii) detection of the viral genome within macrophages (253). The localization of the virus in the cytoplasm of macrophages, but not in phagolysosomes, suggests infection, and not phagocytosis, as the mode of viral entry into macrophages (253).

Recent studies demonstrate that the presence of the L* viral protein allows the DA strain of TMEV to infect and persist in macrophages (320, 483). In vitro studies of TMEV-macrophage interactions show that TMEV preferentially infects activated macrophages (180, 181). Undifferentiated M1 cells were not susceptible to the BeAn strain of TMEV, whereas M1 cells treated with IFN-γ became susceptible to TMEV infection as well as apoptosis (181). In addition, while microglia may play a role in TMEV pathogenesis, viral persistence seems to depend more on blood-borne macrophages. The depletion of blood-borne macrophages by treating SJL mice with dichloromethylene diphosphate leads to a loss of viral persistence as well as a lack of demyelination (398). Time course studies characterizing the persistently infected cells demonstrate that there is an increase in the number of infected cells that are F4/80 positive while the number of infected oligodendrocytes and astrocytes remain constant (398). These results, taken together, suggest that circulating macrophages cross the blood-brain barrier and serve as hosts for persistent TMEV DA infection. It is, however, not yet clear whether (i) the macrophages are sufficiently activated for TMEV infection before crossing the blood-brain barrier, or if such activation of macrophages occurs locally within the CNS, and (ii) what role the L* protein of TMEV-DA plays in infection and persistence.

While TMEV persistence contributes to demyelination, it is thought that autoimmune mechanisms are responsible for triggering the actual demyelinating process itself. The presence of activated macrophages (both infected and uninfected) found in demyelinating lesions suggest that these cells also contribute to the demyelinating process (357). These macrophages express MHC class II molecules and B7-1 and B7-2 costimulatory molecules (357), suggesting that they are presenting antigens to the $CD4^+$ T- cells that are also in close proximity to the demyelinating lesions (357). Recent studies show that macrophages isolated from animals infected with TMEV are able to present to T cells self-antigens, such as proteolipid proteins, in addition to viral antigens (199), suggesting that macrophages can serve as a possible link between immune responses directed against the virus and self.

In addition to activating $CD4^+$ T cells (357), macrophages and microglia are known to produce proteolytic factors that degrade MBP (257). While B6-derived macrophages and microglia infected with the BeAn strain of TMEV had no effect on MBP degradation, macrophages and microglia from SJL mice infected with BeAn released factors that degraded MBP (257). In addition, these factors were found to be cytotoxic to the E20.1 oligodendrocyte cell line (257). Activated macrophages also produce tumor necrosis factor alpha (TNF-α) (138, 455). In transgenic mice where TNF-α expression is induced in the CNS, apoptosis of oligodendrocytes is seen (7), while in TMEV-infected mice, TNF-α has been implicated in the loss of myelin (364). Not surprisingly, high levels of TNF-α have been found in TMEV-infected SJL mice during the chronic demyelinating disease phase by ourselves and others (70, 170). In addition, within the microenvironment of demyelinating lesions in TMEV-infected mice, the presence of foamy (myelin-laden) macrophages has been documented (327). Foamy macrophages are also found in demyelinating lesions of patients with MS (316, 334).

ADHESION MOLECULES

Interactions of lymphocytes with the endothelium are required for lymphocyte trafficking into the CNS. Adhesion molecules play a critical role in determining such interactions and are pivotal in lymphocytes infiltrating the CNS through the blood-brain barrier (103). One such molecule is the intercellular cell adhesion molecule 1 (ICAM-1), a glycoprotein that interacts with many β_2-integrins such as lymphocyte function-associated antigen-1 (LFA-1) (274) on T- cells and CD11b/CD18 (102) on monocytes. Endothelial cells (of the brain-blood barrier) can upregulate ICAM-1 expression upon stimulation by cytokines (189, 349, 464) and/or chemokines (50, 94). However, the involvement of ICAM-1 in TMEV pathogenesis is unclear. In one study, administration of anti-ICAM-1 antibody or simultaneous administration of anti-ICAM-1 antibody with anti-LFA-1 antibody has been shown to be effective in decreasing both the inflammatory response and the demyelinating disease in TMEV-infected mice (171). However, although anti-ICAM-1 antibody treatment has been shown to increase the severity of EAE after induction by adoptive transfer, anti-ICAM-1 did not significantly affect the development of TMEV-induced disease (397). More convincingly, while ICAM-$1^{-/-}$ mice (in the *H-2*^{b} background) develop greater inflammation after TMEV infection than does the wild type, ICAM-$1^{-/-}$ mice are still able to clear the virus and refrain from developing the demyelinating disease (111).

Another important pair of adhesion molecules mediating the interactions between lymphocytes and endothelial cells is

L-selectin (on lymphocytes) and E-selectin (on endothelial cells) (456). However, studies in L-selectin$^{-/-}$ mice showed that while the levels of CD8$^+$ T-cells infiltrating the CNS decreased and the levels of B cell infiltrating the CNS increased, no significant changes in TMEV-induced disease with respect to viral persistence or demyelination was observed (518).

It should be noted that the above studies were focused on single adhesion molecules involved in the interactions of the blood-brain barrier and the pathogenic lymphocytes. Other adhesion molecules not yet studied in TMEV pathogenesis may account for the lymphocyte-endothelium interactions required for lymphocytic trafficking into the CNS. Trafficking mechanisms that depend on other adhesion molecules may be responsible for the results obtained with the adhesion molecule-deficient mice experiments described above (111, 518). For example, very late antigen 4/vascular cell adhesion molecule 1 (VCAM-1) interactions are thought to be pivotal for T-cell migrations into the CNS in experimental autoimmune encephalomyelitis (EAE) (511).

Immunohistochemical analysis of post mortem CNS samples from MS patients showed increased microglia-associated expression of VCAM-1 at the edges of active demyelinating lesions but not in inactive lesions (352). In addition, certain studies have reported an increased presence of soluble ICAM-1 (sICAM-1) in the blood and CSF of MS patients (282, 473), especially in patients with progressive MS (204). Although the significance of the increased expression of sICAM-1 is unknown, it appears that ICAM-1 alone can induce VLA-4 expression in T cells by signaling through CD11a (435). Therefore, although the role of sICAM-1 in MS is unclear, one can envision its involvement in both lymphocyte trafficking and activation.

CD4 AND CD8 T CELLS

Both CD4$^+$ and CD8$^+$ T cells appear to play important roles in the pathogenesis of both early acute disease and late chronic demyelinating disease induced by TMEV. Although the specificity of infiltrating T cells during early acute disease is against the virus, there is limited information about the antigen recognized by infiltrating T cells during late chronic demyelinating disease (190–192, 198, 199, 240, 241). These T cells may recognize host or viral antigens. Identification of the antigenic specificity or specificities of T cells infiltrating the CNS of mice with TMEV-induced late chronic demyelinating disease or of T cells infiltrating brain plaques of patients with MS is, in our view, the most important scientific problem that needs to be resolved in the pathogenesis of demyelinating diseases of the CNS. Both CD4 and CD8 cells contribute to myelin damage (389).

Several lines of evidence demonstrate that CD4$^+$ T cells play an important role in the development of demyelinating disease: (i) treatment of TMEV-infected susceptible SJL mice with anti-CD4 (495) or anti-I-A antibodies (126, 382) resulted in decreased demyelinating disease; (ii) CD4$^+$-mediated delayed-type hypersensitivity (DTH) to TMEV antigens has been associated with myelin damage (291); (iii) while the wild-type B6 mice clear the virus and are resistant to demyelinating disease, TMEV infection of CD4$^{-/-}$ B6 mice results in viral persistence and demyelinating disease (122, 308, 318); (iv) TMEV infection of susceptible SJL mice with genetically deleted CD4 molecules significantly increased the severity of demyelinating disease (308); and (v) the specificity of certain CD4$^+$ T cells generated during the course of TMEV infection induced by either the BeAn or the DA strain of TMEV has been described. These T cells recognize three predominant viral peptides ($VP1_{233–250}$, $VP2_{74–86}$, and $VP3_{24–37}$) (137, 509, 510). It is not fully understood how deficiency in class II gene products contributes to persistence of TMEV and the level of demyelination. The lack of "help" from CD4$^+$ T cells could affect the generation of CD8$^+$ T-cell responses. T-cell help by CD4$^+$ cells is required for antibody production. It has been demonstrated that the levels of specific antiviral antibodies in the class II-deficient mice or mice treated with antibodies to class II gene products is very low in comparison to those in control/immunocompetent TMEV-infected mice (122, 126, 318, 382, 495). Lack of neutralizing antiviral antibodies in CD4$^+$ T-cell-deficient mice may lead to neurological impairement and demyelinating disease by reduction of the clearance of the virus, resulting in an increase in the viral load in oligodendrocytes (122, 126, 318, 382, 495).

Also, several lines of evidence demonstrate that class I-restricted CD8$^+$ T cells play an important role in the development of demyelinating disease: (i) depletion of CD8$^+$ T lymphocytes using an anti-CD8 monoclonal antibody (MAb) greatly reduced myelin destruction in the CNS of TMEV-infected animals (393); (ii) the extent of demyelination was proportional to the expression of H-2 class I expression in the CNS of TMEV-infected mice (393); (iii) TMEV infection of class II-deficient mice results in demyelination (308); (iv) TMEV infection of resistant B6 mice with genetically deleted CD8 resulted in viral persistence and demyelinating disease, suggesting that at least for B6 mice, intact class I- and class II-restricted immune responses are essential for viral clearance (290, 376); (v) demyelination in resistant B6 mice deficient for either the CD4 or the CD8 molecule is preceded by deficient viral clearance (290, 308, 376); (vi) motor functions are preserved in TMEV-infected mice by blocking viral peptide-specific CD8$^+$ T cells with free peptide (188); and (vii) CD8-deficient mice infected with TMEV (BeAn strain) displayed enhanced susceptibility to TMEV infection and increased pathological changes during demyelination (28). The requirement for CD8$^+$ cytotoxic T lymphocytes (CTL) for clearing the virus may be responsible for these results. In contrast, TMEV DA infection of SJL susceptible strains of mice with genetically deleted CD8 molecule does not have any effect on the level of myelin damage (308).

It has been suggested that certain CD8$^+$ T cells may also play a regulatory role in TMEV-infected SJL mice (27, 28, 121, 153, 361). Recent studies by Karls et al. (193) indicated that the susceptibility of BALB/cAnNCr mice to TMEV-induced demyelinating disease is due to the defective function of regulatory CD8$^+$ T cells, which do not receive an activation signal from CD4$^+$ T cells at early stages of infection.

CYTOTOXIC T LYMPHOCYTES

It appears that the development of viral persistence in the CNS of TMEV-infected mice precedes the development of

demyelinating disease (308, 318). The differences in the clearance of the virus between TMEV-infected susceptible strains (such as SJL) and resistant strains (such as B6) leads to demyelinating disease in susceptible strains of mice and to complete recovery in resistant strains of mice. The mechanisms responsible for these differences are not fully understood. Lack of complete viral clearance in TMEV-infected SJL mice results in viral persistence and demyelinating disease which is immunologically mediated. TMEV-infected SCID mice survive TMEV-induced early acute encephalitic disease and do not develop demyelinating disease (389). However, reconstitution of these SCID mice with adoptive transfer of *H-2*-matched splenocytes from immunocompetent mice, results in the development of demyelinating disease in the CNS.

CTL appear to play an important role in the pathogenesis of TMEV-induced disease. Several lines of evidence are supporting the significance of $CD8^+$ CTL in clearing TMEV, including the following: (i) resistance to demyelinating disease in B6 mice is determined by the *H-2D*^{b} locus (22); (ii) passive transfer of $CD8^+$ T lymphocytes from resistant TMEV-infected BALB/cByJ mice to susceptible BALB/cAnNcr animals protected the latter against demyelinating disease but only when the transfer of the cells was carried out early enough to allow clearance of TMEV; and (iii) DBA/2 mice can be protected from TMEV-induced demyelinating disease by in vivo administration of interleukin-2 (IL-2), a growth factor for T cells that may act by recruiting virus-specific CTL (226).

Resistant B6 mice are capable of generating a strong CTL response specific for the VP2 protein of TMEV as early as 3 days p.i. (96, 242, 244). A predominant *H-2D*^{b}-restricted $VP2_{121–130}$ viral peptide in BeAn- or DA-infected mice recognized by CTL has been identified (322, 354). Two other *H-2D*^{b}-restricted TMEV epitopes ($VP_{165–173}$ and $VP_{110–120}$) recognized by CTL have been also described (270). In contrast, only a weak TMEV-specific CTL response is generated in the CNS of TMEV-infected SJL mice (96, 242, 244). Although the appearance of $CD8^+$ T cells infiltrating the CNS of TMEV-infected SJL mice at 11 days p.i. has been reported (243), the function of these $CD8^+$ T cells is not known. They may not be mature CTLs, and they may not exhibit cytolytic activity. TMEV-specific CTL activity appears much later in these mice and remains very low well into the late chronic demyelinating disease phase (up to 180 days p.i., the latest time p.i. when CTL activity has been tested [96]). Furthermore, TMEV-specific CTL precursor frequency in B6 mice has been reported to be relatively high, at 1 in 7,200 and at 1 in 9,000 splenocytes at 10 and 21 days p.i., respectively (96). In contrast, the frequency of TMEV-specific CTL precursors in susceptible SJL mice is low and has been reported to be 1 in 125,000 and 1 in 50,000 splenocytes at 10 and 21 days p.i., respectively (96). The activity of these CTL was determined using appropriate fibroblast cell lines (KSSV [*H-2*^{s}] and C57SV [*H-2*^{b}]) as target cells (241). These results demonstrate that anti-CTL cytotoxic responses appear considerably later in SJL than in B6 mice and remain low throughout the TMEV infection.

The mechanisms that are prohibiting SJL mice, in contrast to B6 mice, from generating a sufficient antiviral CTL response to clear the viruses are not fully understood. We have demonstrated (70) that during early acute disease, transforming growth factor β (TGF-β) transcripts were expressed in the brains of TMEV-infected SJL mice at levels 9 to 10 times higher than those found in the brains of TMEV-infected B6 mice. In addition, TGF-β protein was found only in the CNS of TMEV-infected SJL mice but not in B6 mice. TGF-β was produced by infiltrating mononuclear cells and was found primarily in the leptomeninges of the mesial temporal lobe. TGF-β exhibits strong immunosuppressive properties, which are discussed in the cytokine section of this review (see below). TGF-β is able to greatly inhibit the immune response (reviewed by Roberts and Sporn [377] and Kulkarni and Letterio [215]).

It should be mentioned that the failure to generate a CTL response sufficient to clear the virus in TMEV-infected SJL mice does not appear to be an intrinsic defect of SJL mice. SJL mice were capable of clearing SFV (also a model of virus-induced encephalitis and demyelination) (107, 297). It is not known whether SFV peptides are present in the context of *H-2D*^{s} or *H-2K*^{s}. SFV-infected B6 mice exhibited higher viral titers in the brain, developed more severe early acute disease, and produced lower levels of proinflammatory (Th1) cytokine transcripts (TNF-α, IL-6, and IL-1) and higher levels of anti-inflammatory (Th2) cytokine transcripts (IL-4) than did SFV-infected SJL mice (297).

Kang et al. (191) reported that SJL mice infected with the BeAn strain of TMEV in fact generated a $CD8^+$ cytotoxic TMEV-specific T-cell response directed against the following three *H-2K*^{s}-restricted viral epitopes: a dominant epitope ($VP3_{159–166}$) and two subdominant epitopes ($VP1_{11–20}$ and $VP3_{173–181}$) of the BeAn virus capsid protein (191). The TMEV-BeAn strain is different from the TMEV DA strain, as discussed above (Table 1). The two strains of the virus were discussed in the Introduction. These TMEV-specific CTL produced IFN-γ. A large number of 20-mer peptides with sequences overlapping the major capsid proteins of TMEV BeAn (L1, VP1, VP2, VP3, and VP4) were examined for IFN-γ production by an enzyme-linked immunospot (ELISPOT) assay (191). It was determined that $CD8^+$ CTL infiltrating the CNS of TMEV BeAn-infected SJL mice recognize the following three *H-2K*^{s}-restricted viral epitopes: $VP1_{11–20}$ $VP3_{159–166}$, and $VP3_{173–181}$ (191). These CTL have fully cytotoxic potential and lysed target cells pulsed with appropriate peptides. These T-cell responses were *H-2K*^{s} and not *H-2D*^{s} restricted (191).

In a separate report, Kang et al. (190) compared the avidity and the viral epitopes recognized by $CD8^+$ T cells infiltrating the CNS of SJL mice infected with either the BeAn or the DA strain of TMEV. SJL mice infected with either TMEV BeAn or DA exhibited similar $CD4^+$ T-cell responses to UV-inactivated TMEV BeAn or DA and to the major T-helper epitopes $VP1_{233–250}$ $VP2_{74–86}$ and $VP3_{24–37}$ (137, 190, 509, 510). These VP1 and VP2 T-helper epitopes are identical between the two strains, whereas there is a single amino acid difference in VP3. $CD8^+$ CTL infiltrating the CNS of TMEV BeAn-infected SJL mice recognize the following three *H-2K*^{s}-restricted viral epitopes: $VP1_{11–20}$, $VP3_{159–166}$ and $VP3_{173–181}$ (190). However, $CD8^+$ CTL infiltrating the CNS of TMEV DA-infected SJL mice recognize only one, $VP1_{11–20}$, of these three *H-2K*^{s}-restricted viral epitopes. The peptide sequence differences between the BeAn and DA strains in two of the three predominant and intermediate epitopes do not permit the induction of

$CD8^+$ T cell responses in TMEV DA-infected SJL mice (190). Similar results were obtained with IFN-γ determinations by ELISPOT assay and intracellular cytokine staining (190). Although the response to the $VP1_{11–20}$ epitope alone in TMEV DA-infected SJL mice is higher than that in BeAn-infected SJL mice, the overall response of the $CD8^+$ T cells infiltrating the CNS of DA-infected SJL mice is substantially lower than that observed in BeAn-infected SJL mice because of the lack of response to the $VP3_{159–166}$ and $VP3_{173–181}$ DA epitopes. *H-2K*^{s} but not *H-2D*^{s} restriction of virus-specific cytotoxicity in the CNS of TMEV DA-infected mice has also been reported by other groups (240, 241).

The discrepancy between these results and those reported by other groups describing the low level and late appearance of CTL in SJL mice could be attributed to the fibroblast cell line (KSSV [*H-2*^{s}]) routinely used as target cells in these CTL experiments (97, 241, 242, 244). This cell line expresses low level of *H-2K*^{s}. In contrast, Kang et al. (190, 191) used as target cells the EL-4 cell line transfected with expression constructs for *H-2K*^{s}, achieving much higher *H-2K*^{s} expression. However, it is not clear which of the two targets (high versus low expressor) approximates most closely the in vivo situation. The expression of *H-2* class I is weak in normal brains. However, IFN-γ produced in response to viral infection most probably enhances the expression of both class I and class II MHC on neurons, oligodendrocytes, and astrocytes (71, 120, 373).

The significance of *H-2K*-restricted virus-specific cytotoxicity in TMEV-infected SJL mice has been emphasized by demonstrating the down regulation of this *H-2K* molecule by L* (241). The TMEV DA L* protein is a Zn metalloprotein, and Zn binds to a Cys-His motif which is conserved among cardioviruses (74). The mutant virus DA L*-1 (which does not synthesize L* protein) induces *H-2K*^{s}-virus-specific cytotoxicity and (in contrast to wild-type DA virus) does not persist in the CNS of infected mice.

CLONALLY EXPANDED T CELLS ARE PRESENT IN THE CNS OF TMEV-INFECTED MICE AND IN THE CENTRAL NERVOUS SYSTEM OF PATIENTS WITH MULTIPLE SCLEROSIS

TMEV-Infected Mice

Studies to determine the presence of clonally expanded T cells in the CNS of TMEV-infected mice have been carried out using size spectratyping of β-chain TCR transcripts followed by sequencing of the CDR3 region. Clonal expansions were observed in the CNS as early as 7 days p.i. and remained there throughout the course of the late chronic demyelinating disease (192). However, the T-cell repertoire during the early acute disease (7 to 9 days p.i.) appeared to be more diverse than that observed during late chronic demyelinating disease (65 days p.i.) (192). The vast majority of the T-cell clones that were found to be clonally expanded during early acute disease were also found to be clonally expanded during late chronic demyelinating disease (192). Clonal expansions were not detected in the CNS of SJL mice with proteolipid protein (PLP)-induced EAE. We have also found clonally expanded β-chain TCR transcripts in the spinal cord of TMEV-infected SJL mice 180 days p.i. (E. L. Oleszak, W. L. Lin, J. R. Chang, and C. D. Platsoucas, unpublished data). We observed identical clonally expanded transcripts in the spinal cords of a substantial number of TMEV-infected SJL mice studied. We also observed clonally expanded β-chain TCR transcripts present only in individual mice. These results are in contrast to those of Rodriguez et al. (391), who found no preferential expression of TCR β-chains in the CNS of TMEV-infected SJL mice with prominent demyelination.

Johnson et al (187) employed D^b:$VP2_{121–130}$ peptide tetramers to stain $CD8^+$ CNS-infiltrating lymphocytes from the spinal cords of TMEV-infected C57BL/6 mice. They found that 50 to 63% of these cells expressed TCR specific for the $VP2_{121–130}$ peptide presented in the context of D^b. However, T cells expressing this TCR were totally absent from the cervical lymph nodes and spleen of these TMEV-infected SJL mice. The $VP2_{121–130}$ epitope presented in the context of D^b appears to be an immunodominant epitope in TMEV-induced disease.

Studies by Bahk et al (24) seem to link susceptibility of SJL mice to TMEV-induced demyelination with restriction fragment length polymorphism of the Jβ1-Cβ1 region and not with that of the Vβ1 region. When the MHC haplotype was also included in the linkage analysis, the association was even stronger.

Oligoclonal T Cells Are Present in Brain Plaques from Patients with MS

Studies to determine whether T cells infiltrating brain plaques or extravasating into the cerebrospinal fluid (CSF) of patients with MS contain oligoclonal populations of T cells have shown: (i) the presence of clonally expanded α- and β-chain TCR transcripts from chronic MS plaques (323, 436), CSF (149, 231), or peripheral blood (309) from patients with MS, and (ii) predominant or preferential utilization of particular Vα or Vβ TCR gene segments employed by T cells infiltrating chronic MS plaques (40, 323, 324). In one study, TCR transcripts were found that rearranged only 0 to 9 (4.4 ± 2.0 [mean ± standard deviation) different Vα gene segments (primarily Vα1, Vα2, Vα7, Vα8, and Vα10) and 2 to 13 (7.0 ± 3) different Vβ gene segments (primarily Vβ5.2/5.3, Vβ6, Vβ7, Vβ8, and Vβ12) per brain of patients with MS (323).

Oksenberg et al (323) reported that TCR transcripts utilizing Vβ5.2/5.3 were found in the brains of all patients examined who carried the HLA DRB1*1501, DQA1*0102, DQB1*0602, and DPB1*0401 alleles. Five CDR3 motifs were identified (323). One of these motifs was identical to that found in an MBP-specific human Vβ5.2 T-cell clone that exhibited cytotoxicity against target cells presenting MBP (89–106) (276). The amino acid CDR3 motif Leu-Arg-Gly of this human T-cell clone was identical to a rat T-cell clone, isolated from the brain of a rat with EAE, that was specific for MBP (87–99) (142). Forty percent (16 of 40) of the Vβ5.2/5.3 rearrangements from MS lesions were specific for MBP, demonstrating that a major portion of the $V\beta5.2/5.3^+$ T cells had mounted an immune response to MBP (323). These MBP-specific Vβ5.2/5.3 transcripts represent a small proportion (less than 10%) of the total β-chain transcripts found in plaques of patients with chronic MS.

MBP-specific T-cell clones from peripheral blood leukocytes of DR2-positive patients with MS preferentially utilize the

Vβ5.3 gene segment (214). One of these patients utilized the LR motif in the CDR3 (214) that was found in chronic plaques of patients with MS (323) and a human T-cell clone specific for MBP (89–106) (276). However, there is no information about TCR transcripts of T cells infiltrating MS plaques from DR2-negative patients. Musette et al. (309) reported the presence of an oligoclonal $CD4^+$ T-cell subset in the peripheral blood of DR2-positive patients with MS, which utilizes Vβ5.3 and Jβ1.4 gene segments; this population is present, but not expanded, in DR2-positive normal donors but completely absent from DR2-negative patients with MS or DR2-negative normal donors.

Other Vβ genes may also be involved in MS. Hong et al. (159) have identified a CDR3 motif, Vβ13.1-LGRAGLTY, which was found in several MBP-reactive $V\beta13.1^+$ T-cell clones derived from a number of patients with MS.

Recently we have detected the presence of oligoclonal T cells in the CSF of a child with MS-like disease following hepatitis A infection (332). MRI findings indicated the presence of multiple metachronous demyelinating lesions predominantly in the white matter that were widely spread throughout the CNS within 5 days after the onset of neurological symptoms. Hepatitis A virus is a picornavirus. It is not clear whether clonally expanded T cells in the CSF of this patient are specific for a self-peptide or for a hepatitis A virus determinant.

Hafler et al. (150) reported the absence of an association of particular Vβ and Jβ gene segments with MS. TCR transcript sequences were provided for comparison by 17 different laboratories. Comparison of CDR3 motif frequencies in (i) human MBP-reactive clones, (ii) MS-brain-derived clones of undefined specificity, and (iii) control clones of all other specificities and origins did not reveal significant differences, even when functionally identical amino acids were treated as equivalent. Since MHC restrictions were not taken into consideration, further examination of CDR3 motifs in patients with MS may be warranted.

COSTIMULATORY MOLECULES

Role in the Development of TMEV-Induced Disease

In addition to TCR-antigen-MHC engagement, a second, or costimulatory, signal is required for the productive activation of naive T-cells. The second signal usually comes in the form of the CD28 molecule interacting with B7.1 or B7.2 on antigen-presenting cells (APC) such as macrophages, B cells, and dendritic cells (11). CTLA-4 is thought to be important in regulating T-cell responses (11). In addition to macrophages and dendritic cells that migrate into the CNS from the periphery, microglia and astrocytes are capable of both presenting antigens (198, 199, 335) and providing costimulatory signals (80, 500). In TMEV-induced demyelinating disease, costimulatory molecules are necessary for the generation of CTLs and for the anti-TMEV antibody responses required for clearing the virus (315). Blocking of the CD28-B7 interaction by CTLA-4–immunoglobulin (Ig) or by simultaneous administration of anti-B7.1 and anti-B7.2 antibodies during TMEV infection induces exacerbated disease, as evidenced by increased clinical symptoms (315). In addition, $CD28^{-/-}$ mice (187) or $CD40L^{-/-}$ mice (110) on the resistant *H-2*^{b} background are susceptible to TMEV-induced late chronic demyelinating disease. Also, the CTLA-4–B7 interaction induces the production of indoleamine-2,3-deoxygenase through an IFN-γ-dependent pathway in macrophages and dendritic cells (146). The products of tryptophan catabolism such as hydroxyanthranylic acid and quinolinic acid are neurotoxic (reviewed by Chiarugi et al. [75] and Look et al. [259]). In addition, CTLA-4 induced neurotoxin production may contribute to the CNS damage.

Role in MS

In patients with progressive MS, B7.1 expression was increased in $CD4^+$ cells (500) while B7.2 expression was seen in both $CD4^+$ and $CD8^+$ T cells (286). Treatment of MS patients with IFN-β resulted in a lower expression of B7.1 molecules on B lymphocytes and an increased expression of B7.2 molecules on monocytes (256). In addition, soluble CD28 and soluble CTLA-4 molecules have been found in the serum of IFN-β-treated MS patients (139), suggesting that one mechanism by which IFN-β ameliorates MS symptoms is by suppressing the interactions of the costimulatory molecules. However, the numbers of $CD4^+$ $CD28^-$ T cells autoreactive to MBP were expanded in MS patients (271). This supports an earlier finding that autoreactive T cells were capable of expanding in the absence of B7 costimulation (418). $CD4^+$ $CD28^-$ T cells are resistant to apoptosis (271) and have a Th1 phenotype (497). In MS, these cells produced IFN-γ and IL-12Rβ2 without costimulation (271). Although it appears that CD28 is important during either the onset or the progression of MS, the exact role of $CD4^+$ $CD28^-$ T cells is unclear. One possibility is that prolonged stimulation of these cells may be a mechanism for the production of additional inflammatory cytokines.

EPITOPE SPREADING, MOLECULAR MIMICRY, AND AUTOIMMUNITY

TMEV-Induced Disease in Susceptible Mice

As discussed above, both $CD4^+$ and $CD8^+$ cells contribute to demyelinating disease in TMEV-infected mice. It has been suggested that in the TMEV BeAn-infected mice the virus triggers predominantly Th1 proinflammatory antiviral responses, which in turn lead to the development of additional T-cell responses to myelin epitopes. This phenomenon is known as epitope spreading, and it has been described in a number of autoimmune diseases (199, 233, 293, 294, 482). Epitope spreading is an acquisition of neoreactivity which results from endogenous priming with self antigens generated from damaged tissue over the course of the disease (232, 427). Antiviral $CD4^+$ and $CD8^+$ responses appear early after TMEV infection (within the first week) and persist throughout the life of the animals (detected as late as 300 days p.i.). In contrast, myelin-specific T-cell responses appear at about 50 to 60 days p.i. and also persist in infected animals (199, 233, 482). Myelin epitopes are presented by $F4/80^+$, $I\text{-}A^s$, $CD45^+$ macrophages and microglia to T cells (294, 482). Miller et al. (296) reported that in TMEV BeAn-infected mice, specific T-cell responses were restricted predominantly to the PLP (139–151) epitope, which appeared 54 to 60 days p.i. These TMEV BeAn-infected mice exhibited clinical symptoms (gait disturbance, hind leg weakness, and abnormal righing reflex) at 30 to 40 days p.i. strongly suggesting that PLP-specific T-cell re-

sponses are not responsible for the induction of the disease but that they may participate in propagation of the disease through epitope spreading. These PLP(139–151) T-cell responses do not arise because of cross-reactivity between TMEV and myelin epitopes (294). They are generated in the process of de novo priming of self-reactive T cells to self antigens released due to bystander myelin destruction, perhaps by virus-specific T cells, in the setting of chronic inflammation. These results suggest that late chronic demyelinating disease in TMEV-infected mice is not initiated by epitope spreading, but may be propagated by T-cell responses generated through epitope spreading (199, 233, 294, 482).

At about 3 months p.i., in addition to viral epitopes, other epitopes of PLP were presented by APC from the CNS of TMEV-infected mice, including PLP (56–70), PLP (104–117), and PLP (178–191) (199). DTH responses to MOG(92–106) and to MBP(84–104) have also been detected late in the course of demyelinating disease (199). Studies by Dal Canto et al. revealed that epitope spreading has functional activity, because lymphocytes from mice chronically infected with TMEV produced demyelination of organotypic cultures after stimulation with the major encephalitogenic peptide of PLP (85). Therefore, late chronic demyelinating disease induced by TMEV illustrates how viral infection of the host may result in autoimmune disease through the generation of epitope spreading. It should be noted that naive SJL mice have a very high frequency of PLP(139–151)-reactive T cells in the periphery and that they may be particularly susceptible to autoimmune disease (15).

Epitope spreading may not be the only factor contributing to autoimmunity in TMEV-infected mice. Molecular mimicry is another mechanism that may contribute to the generation of autoimmune disease in these mice. Molecular mimicry is defined as the presence of common epitopes between host proteins and microorganisms such as bacteria and viruses (130, 241, 506). Under these circumstances, an immune response of the host to a viral epitope will recognize, as nonself, the cross-reacting host epitope even when the virus or the microorganism is no longer present. This may lead to the development of autoimmune disease. Along these lines, a molecular mimicry has been reported between anti-TMEV antibody responses in TMEV-infected mice and the myelin component galactocerebroside (reviewed by Tsunoda and Fujinami [475]). Recently, Tsunoda et al. reported the induction of autoreactive $CD8^+$ cytotoxic T cells in TMEV DA-infected mice (476). They demonstrated that spleen cells from TMEV-infected mice collected at 3 weeks p.i. and stimulated in vitro with TMEV-infected APC exhibited high level of cytotoxicity to uninfected syngeneic target cells, probably by molecular mimicry, but not against allogeneic cells i.e. inoculation of these effector cells into naive mice resulted in inflammatory lesions in the spinal cord.

MS in Humans

Autoimmunity is the response of the immune system (either B or T cells) to self-components. MS is postulated to be a cell-mediated autoimmune disease directed against CNS components. However, it is not clear whether this autoimmune disease is triggered by initial viral infection. The way in which viruses could trigger an autoimmune disease by molecular mimicry has been addressed by Wucherpfennig and Strominger (506). Recent studies indicate that MBP is one of the most important targets in the immunopathogenesis of MS. It has been documented that the MBP(85–99) peptide is a T-cell target for patients with the HLA-DR2 haplotype while the MBP(88–102) peptide may be a target for patients with other HLA-DR haplotypes. Peptide-binding studies determined which MBP peptide residues were important for binding to DR2 and which ones were necessary for binding to TCR. These criteria have been applied to generate a minimal molecular mimicry peptide. This structural motif has been used to search a sequence database for viral and bacterial mimicry peptides of MBP(85–99). The search yielded 129 viral and bacterial peptides, 8 of which had biological activity and stimulated MBP(85–99)-specific T-cell clones. These peptides did not show any significant linear homology to MBP(85–99), and they were derived from common human pathogens (EBV, herpes simplex virus cytomegalovirus, influenza virus, and adenovirus). These elegant studies illustrated how a common virus may trigger the autoimmune process (504).

Molecular mimicry (see the previous section) may play a role in the pathogenesis of MS. Recently, Lang et al (225) provided structural evidence for molecular mimicry involving HLA, EBV, and MBP. Crystal structure determination of the DRB1*0101 and EBV peptide showed significant similarities to the complex of the DRB5*0101-MBP peptide at the surface presented for TCR recognition (225). Molecular mimicry involving microbial peptides and myelin antigens in patients with MS has been recently reviewed (239, 275, 513). It is possible that in patients with MS, molecular mimicry may involve additional targets other than MBP, such as PLP and MOG (33, 203, 338, 493). We have previously shown molecular mimicry between the S peplomer protein of the mouse hepatitis virus JHM, another model of demyelinating disease, and the Fcγ receptor (325, 329, 330, 333).

Epitope spreading has been also recognized in patients with MS. Studies by Tuohy et al (478) have indicated that progression in patients with MS involves shifting of autoreactivity from primary self-peptides to a number of secondary determinants. However, the dynamics of the appearance of new self-epitopes and their role in the propagation of demyelinating disease are not clear (141, 480, 522).

ROLE OF CYTOKINES

TMEV-Induced Disease in Mice

Cytokines play a critical role in the induction and regulation of the immune response to neurotropic and other viruses. Cytokines and chemokines may play a critical role in the pathogenesis of these viral infections and in persistent infections. Clearance of the virus from the CNS requires the migration of cells of the immune system to the site of infection and involves specific antigen-driven proliferation and differentiation of the cells of the immune system to antiviral effector cells (124, 277, 350). All of these processes are dependent on, or heavily influenced by, cytokines and chemokines. The immune response to TMEV infection is heavily influenced by the production of cytokines both by the inflammatory cells of the immune system,

such as T cells and macrophages, and by nonimmune cells of the CNS, such as astrocytes and microglia. Cytokines are essential not only for enhancing and propagating antiviral immune responses resulting in clearance of the virus but also for downregulating these responses after clearance of the virus. Several investigators, including ourselves (28, 70, 408, 460), investigated the role of cytokines in TMEV-induced neurological disease. In contrast to our studies (70), a number of laboratories (28, 408, 460) have examined the expression of cytokine transcripts in the CNS of susceptible and resistant strains of mice, infected with either the DA strain or the BeAn strains of TMEV, without successfully identifying particular cytokine patterns or cytokines that could be associated with resistance or susceptibility to TMEV-induced encephalomyelitis or demyelinating disease or with the inability of susceptible strains of mice, such as SJL, to clear the virus during early acute disease.

Sato et al. (408) found mostly Th1 cytokine transcripts in the CNS of TMEV DA-infected mice, while certain Th2 cytokine transcripts were also expressed. Individual cytokines or cytokine patterns were not identified in this study as being associated with susceptibility or resistance to early acute or late chronic demyelinating disease induced by the virus or with the inability of SJL mice to clear the virus, possibly because only four time points were examined during the course of the disease. This design may be responsible for the lack of identification of particular cytokines or cytokine patterns associated with the disease.

Begolka et al. (28) used a different strain of TMEV, the BeAn strain, whereas we (70) and Sato et al. (408) used the DA strain. The BeAn strain induces a substantially different disease. BeAn-induced early acute disease is more attenuated than that induced by the DA strain. The onset of the clinical symptoms associated with demyelinating disease induced by the BeAn strain can be documented as early as 30 days p.i. (175, 199, 294). Begolka et al. (28) found that during BeAn-induced disease in SJL mice, the levels of IFN-γ and TNF-α transcripts correlated well with maximum clinical activity whereas IL-4 and IL-10 transcripts were found throughout the course of the disease (28).

Theil et al. (460) compared the abilities of three neurovirulent strains of TMEV, DA, GDVII, and H101, to induce the expression of chemokines and cytokines in the CNS of SJL mice. Infection of susceptible strains of mice with these strains of TMEV results in very different disease and neuropathological changes. Similar patterns of expression of chemokine transcripts were observed after infection of SJL mice with each one of the three strains. The following chemokine transcripts were studied: RANTES, monocyte chemotactic protein 1 (MCP-1), IP-10, macrophage inflammatory protein 1β (MIP-1β), MIP-1α, and MIP-2. Differences were found in the levels of cytokine transcripts in the CNS of SJL mice infected with different strains of TMEV. IFN-β and IL-6 transcripts were expressed in high levels in the CNS of SJL mice infected with the GDVII strain of TMEV. High levels of LT-α transcripts were found only during infection with the DA strain of TMEV (460). The authors concluded that proinflammatory cytokines are involved in the pathogenesis of the TMEV-induced disease and modulate the acute and chronic phases of the disease which are characterized by different pathological features.

TABLE 3. Levels of cytokine transcript in the CNS of TMEV-infected susceptible (SJL) and resistant (C57BL/6) mice[a]

Cytokine	Level[b] at:			
	8–14 days p.i.[c] (early acute disease)		39–60 days p.i.[d] (late demyelinating disease)[e]	
	SJL mice	C57BL/6 mice	SJL mice	C57BL/6 mice
TGF-β	High	Low	High	Low
IL-1	High	High	High	Low
IL-6	High	High	High	Low
TNF-α	High	Low	High	Low
IFN-γ	High	High	High	Low
IL-2	Low	Low	Low	Low
IL-12	High	High	High	Low
IL-10	High	High	High	Low
IL-4	Low	Low	High	Low

[a] Data from reference 70.
[b] High, >100% greater than the basal levels seen in mock-infected mice; Low, <50% greater than the basal levels seen in mock-infected mice.
[c] Cytokine transcript levels in the brains of TMEV-infected mice.
[d] Cytokine transcript levels in the spinal cords of TMEV-infected mice.
[e] Observed only in SJL mice.

Primary astrocytes are capable of producing proinflammatory chemokines RANTES and IP-10 on activation by IFN-γ (342) or of producing RANTES on activation by TNF-α (342). Additionally, as observed with cytokine transcript expression (70), on infection with TMEV, mice susceptible to chronic demyelinating disease upregulate RANTES, IP-10, and MCP-1 in a biphasic manner (307) that correlates with TMEV pathogenesis. Interestingly, the increased chemokine expressions during late chronic demyelinating disease were more dependent on the level of viral persistence than on the level of $CD4^+$ or $CD8^+$ T cells (371).

We have reported (70) certain significant differences in the expression of cytokine transcripts and, in certain cases, proteins in the CNS of TMEV DA-infected susceptible (SJL) and resistant (B6) mice during early acute disease and late chronic demyelinating disease at 8, 14, 21, 25, 33, 40, and 60 days p.i. High levels of proinflammatory cytokine transcripts (IFN-γ, TNF-α, IL-1, IL-2, and IL-6) and low levels of anti-inflammatory cytokine transcripts (IL-4, IL-5, and IL-10) were expressed in the brains of TMEV-infected SJL and B6 mice during early acute disease. In TMEV-infected SJL mice, cytokine transcript levels exhibited a biphasic profile, with high levels during early acute disease, reaching a maximum on days 8 to 12 p.i., decreasing thereafter, reaching a minimum around days 20 to 25 p.i., in agreement with the end of the early acute disease and with the disappearance of mononuclear infiltrates from the brain, and then increasing again in the spinal cord of SJL mice. The proinflammatory cytokine response is required to clear the viral infection.

The expression of various cytokine transcripts and their levels in the CNS of TMEV-infected susceptible (SJL) and resistant (B6) mice during early acute disease and late chronic demyelinating disease is summarized in Table 3.

Similarly, a proinflammatory cytokine response has been found in the CNS after infection with a number of neurotropic viruses including Borna disease virus (152), herpes simplex virus (134), SFV (107), and vesicular stomatitis virus (339) infections of the CNS.

TGF-β. As discussed above, we have determined that during early acute disease, TGF-β transcripts were expressed at levels 9 to 10 times higher in the brains of TMEV-infected SJL mice than in the brains of TMEV-infected B6 mice (70). Additionally, TGF-β protein was found in the brains of TMEV-infected susceptible SJL mice during early acute disease but was absent from the brains of TMEV-infected resistant B6 mice (70). Immunohistochemical studies revealed that TGF-β was produced only by infiltrating mononuclear cells and was located mostly in the leptomeninges of the mesial temporal lobe.

TGF-β is a complex immunoregulatory molecule with strong immunosuppressive properties and is able to impair greatly the antiviral immune response (reviewed in references 215 and 377). TGF-β may be responsible, at least in part, for the delayed appearance of TMEV-specific CTL and their low activity in SJL mice. It inhibits the development of CTL (see below). Clearance of the virus requires a prompt and strong CTL response. Lack or delay of this response may be responsible for the failure to clear the virus, permitting the establishment of persistent infection. Late chronic demyelinating disease is the final outcome of this process. In particular, TGF-β inhibits (i) the development of CTL, in particular the differentiation of $CD8^+$ precytotoxic T cells into effector $CD8^+$ CTL (125, 370, reviewed in reference 355); (ii) T-cell proliferation (200, 417), possibly by inhibiting IL-2 production or possibly in connection with other cytokines such as IL-10 (258); (iii) the expression of class II MHC on APC (261, 417); (iv) differentiation of $CD4^+$ cells into Th1 or Th2 types (405, 451); (v) activation and differentiation of APC such as monocyte/macrophages (95, 488), dendritic cells (135, 147), and glial cells, often by blocking the production of reactive oxygen intermediates and nitric oxide production (450); (vi) production of several cytokines, including IL-1, TNF-α, IL-6, and IFN-γ, by activated microglia (417); and (vii) generation of lymphokine-activated killer cells (216).

TGF-β is also produced during late chronic demyelinating disease; however, its role appears to be very different from that in early acute disease. TGF-β may play an important role in the pathogenesis of a number of autoimmune diseases, in particular during the resolution phase, presumably by inhibiting or even eliminating inappropriate or aberrant inflammatory responses and restoring homeostasis after viral clearance. TGF-β has a beneficial effect on several autoimmune diseases in experimental animals. TGF-β treatment significantly improved the clinical course of EAE (288, 366) and diabetes (301) and prevented collagen-induced arthritis (462) and thyroiditis (421). TGF-β has been suggested as a putative treatment for patients with MS (59). Notwithstanding the possible beneficial role of TGF-β in resolving late-phase disease and inhibiting autoimmune responses, its presence during the viral encephalitis stage of the disease (early acute disease) may be detrimental to the host by inhibiting the generation of antiviral CTL and by downregulating the activation of microglia or macrophages, leading to inefficient viral clearance.

IL-1, IL-6, and TNF-α. During early acute disease, high levels of IL-1, IL-6, and TNF-α transcripts (the so-called "inflammatory triad") were found in the CNS of both susceptible and resistant TMEV-infected strains of mice (70). These cytokines are produced locally in the CNS during early acute disease, very probably by both infiltrating mononuclear cells and intrinsic brain cells such as microglia and astrocytes (13, 31, 140, 155, 401, 411, 420, 437). These cytokines contribute to the activation and recruitment of T and B cells and monocytes to the CNS by upregulatng the expression of MHC class I and II and of adhesion molecules on endothelial cells, astrocytes, and microglia (reviewed in references 277, 350, and 415). IL-6 (492, 519), IFN-γ (58, 196, 235, 285), and TNF-α (62, 173, 501) exhibit direct antiviral activity and theoretically are able to directly inhibit viral replication. However, these cytokines alone are not sufficient to completely eliminate the virus from the CNS.

IL-1 is a multifunctional cytokine and is produced primarily by activated cells of the monocyte/macrophage lineage (104). It costimulates T- and B-cell responses (reviewed by Dinarello [105]) and is involved in induction of the expression of adhesion molecules and chemokines (67, 415). In addition, it may be critical in inducing demyelinating disease in TMEV-infected resistant B6 mice which have been treated with lipopolysaccharide (362). It has also been suggested that the ability of glial cells to produce IL-1 in vitro, in response to TMEV infection, is associated with a susceptibility to demyelination (400).

IL-6 was originally designated IFN-β and exhibits direct antiviral activity. Also, it augments the generation of class I-restricted CTL (130, 295). It may also exhibit anti-inflammatory properties, because it inhibits TNF-α production induced by LPS (5) and induces the production of the IL-1 receptor antagonist (463). Administration of recombinant IL-6 to TMEV-infected SJL mice protected these mice against demyelinating disease (388). There were no significant differences in the transcript levels of IL-1 or IL-6 found in the CNS of TMEV-infected SJL or B6 mice (70).

During early acute disease, TMEV-infected SJL mice express much higher levels of TNF-α transcripts in the brain and spinal cord than do B6 mice (70). TNF-α is a strong inducer of inflammatory cytokines and has been reported to be directly cytotoxic to various cell types (reviewed in references 465, 486, and 516) and to cause direct damage to oligodendrocytes in the brains of MS patients (424). TNF-α upregulates the expression of MHC class I and of adhesion molecules on endothelial cells and enhances the recruitment of leukocytes to the CNS (66, 71). Earlier studies demonstrated a clear correlation between demyelination and the level of TNF-α in EAE (28, 89, 374, 402, 422) and MS (369, 375, 431). Inoue et al. reported a correlation between the level of TNF-α and the degree of demyelination in mice infected with TMEV (170). In contrast to these findings, where increased levels of TNF-α appear to promote demyelinating disease, the following should be noted: (i) treatment of TMEV-infected SJL mice with recombinant TNF-α significantly reduced the severity of demyelinating disease (347); (ii) TNF-α protected mice from developing lupus erythematosus (143, 177) and diabetes (176, 365, 410); and (iii) TNF-α-deficient mice developed substantially more severe demyelinating disease after immunization with MOG than did their $TNF\text{-}\alpha^{+/+}$ counterparts (255). These results suggest a dual role for TNF-α, which may exhibit both proinflammatory and anti-inflammatory properties and may play a regulatory role in maintaining host homeostasis.

IFN-γ. The levels of IFN-γ transcripts are higher in the CNS of TMEV-infected SJL mice than in the CNS of B6 mice (70).

The levels of IFN-γ transcripts are substantially increased in the brains of TMEV-infected SJL mice between 15 and 25 days p.i., suggesting additional activation of microglia/macrophages by IFN-γ. These cells may be largely responsible for the partial clearance of the virus from the CNS of TMEV-infected SJL mice. As previously mentioned, SJL mice mount a deficient anti-TMEV CTL response, suggesting that cells of the microglia and macrophage lineage may be important in partially clearing the virus (70). IFN-γ induces and augments MHC class I and II expression by professional and nonprofessional APC, facilitating the presentation of viral antigen(s) to $CD4^+$ or $CD8^+$ T cells by these APC. IFN-γ is also responsible for activating macrophages, microglia, and astrocytes (32, 65, 79, 115, 481), which in turn produce TNF-α, IL-1, and IL-6 as well as nitric oxide (NO) and reactive oxygen intermediates (72, 81, 229, 230, 287, 420). IFN-γ promotes the differentiation of Th1-type cells and inhibits the differentiation of Th2-type cells (303). The significance of IFN-γ in the pathogenesis of TMEV-induced disease has been shown by the following observations: (i) TMEV infection of IFN-γ receptor-deficient mice of resistant background resulted in prominent inflammatory demyelinating disease and (ii) treatment of TMEV-infected SJL/J (susceptible) or C57BL/10SNJ (resistant) mice with anti-IFN-γ MAb substantially increased demyelination (387). IFN-γ is essential for the generation of a sufficiently strong immune response to clear the virus in both resistant and susceptible strains of mice. Alternatively, Pullen et al. (362) suggested that IFN-γ instead plays a regulatory role in TMEV infection.

IL-2. High levels of IL-2 transcripts appeared in the spinal cord of TMEV-infected SJL, but not B6, mice before the onset of the demyelinating phase of the disease, at 25 days p.i. (70). The level of IL-2 transcripts reached a maximum on day 35 p.i. and then declined by day 39 p.i. During all other times, the levels of IL-2 transcripts remained low in the CNS of both TMEV-infected SJL and B6 mice. This increase in the levels of IL-2 transcripts at 25 to 39 days p.i. was accompanied by a noticeable increase in the proportions of $CD3^+$ $IL\text{-}2^+$ T cells infiltrating the spinal cords of TMEV-infected SJL mice which expressed IL-2 protein with respect to the proportions of $CD3^+$ $IL\text{-}2^+$ T cells infiltrating the brains of TMEV-infected SJL mice at 8 days p.i. (70). It is possible that the increase in IL-2 transcripts and protein which we observed in the spinal cords of TMEV-infected SJL mice just before the onset of the demyelinating disease between days 25 and 40 p.i. may be responsible for the expansion of T cells of unknown specificity, which may be responsible, at least in part, for the development of demyelinating disease in the spinal cords of TMEV-infected SJL mice (70). In addition to acting on the cells of the immune system and causing activation and cell division of T, B, and NK cells and macrophages (438), IL-2 has been reported to modulate myelin gene expression in oligodendrocytes (30, 428). Also, IL-2 or an IL-2-like molecule, isolated from the optic nerve of the fish, can inhibit the proliferation of oligodendrocytes or can be directly cytotoxic to oligodendrocytes (113, 114).

IL-12. IL-12 is a heterodimeric cytokine essential for the generation of Th1 cells, is produced by macrophages and dendritic cells, and is composed of two subunits with molecular masses of 35,000 Da (p35) and 40,000 Da (p40) (470, 471). The p35 subunit is constitutively expressed essentially in all tissues, whereas the p40 subunit is expressed in general in cells that produce IL-12 (470, 471). There were no significant differences in the levels of the IL-12 p40 transcripts in the CNS of TMEV-infected SJL and B6 mice (70). IL-12 mediates both innate and acquired immunity, including augmentation of CTL responses and IFN-γ and other Th1 cytokine production during parasitic and viral infections (36, 83, 154, 162, 336). Treatment of TMEV BeAn-infected SJL mice with anti-IL-12 MAb at the time of infection did not affect the level of demyelination and inflammation at 80 days p.i. (172). Similarly, treatment of TMEV DA-infected SJL mice with anti-IL-12 MAb on days 0, 7, 14, 21, and 28 p.i. did not significantly affect the demyelination or the degree of parenchymal inflammation in these mice (49). However, treatment of TMEV-infected SJL mice with anti-IL-12 antibody on days 20, 22, and 26 p.i., long after viral clearance, suppressed demyelinating disease (172). Similarly, the symptoms of many autoimmune diseases that are likely to be associated with the production of high levels of Th1 cytokines are ameliorated in part by treatment with anti-IL-12 antibody (132, 163, 237, 314, 469). Nevertheless, it remains to be established whether IL-12 administration in vivo to TMEV-infected SJL mice would achieve complete clearance of the virus. It has been reported that IL-12 may not be required for the induction of Th1 responses in viral infections (339).

IL-10. There were no significant differences in the levels of IL-10 transcripts in the CNS of TMEV-infected mice (SJL or B6) during early acute disease (70). IL-10 is a Th2 cytokine in the mouse, produced by cells of the monocyte/macrophage lineage as well as by T and B lymphocytes (reviewed by Howard et al. [160], Mosmann [302], and Mosmann and Moore [304]). IL-10 is a cytokine with potent immunosuppressive properties, which include inhibition of (i) antigen-specific proliferation of T cells by downregulating on APC MHC class II expression (54, 212); (ii) production of Th1 cytokines by T cells and monocytes (54, 99, 123, 212); (iii) proliferation of T cells to polyclonal activators (258, 452) and $CD4^+$ T-cell clones, probably by inhibiting the synthesis of IL-2 production (100); (iv) activation of cells of the monocyte/macrophage lineage (46, 106); and (v) transport-associated protein-mediated HLA class I antigen presentation (515). The expression of IL-10 transcripts in mice with EAE has been correlated with recovery (201). In general, administration of IL-10 to rats prevented the induction of EAE (34, 84, 278, 399). However, IL-10 treatment of rats with EAE resulted in exacerbation of the disease (63). The final effect of IL-10 on EAE is far more complicated and may depend on the interactions of both Th1 and Th2 cytokines.

Studies with a nonpathogenic variant of the BeAn strain of TMEV (M2) revealed that in contrast to the parental BeAn strain of TMEV, infection of susceptible SJL mice with the M2 variant of TMEV resulted in the preferential expression of IL-10 over IL-12 by dendritic cells and macrophages (343). Remarkably, because of only one amino acid substitution in the capsid region ($VP1_{Lys244Arg}$) of the M2 variant with respect to the parental BeAn strain of TMEV (343), infection with M2 does not lead to demyelinating disease (343). Furthermore, these findings suggest that the immunosuppressive properties of IL-10 may inhibit the activation of autoreactive T cells.

IL-4. Very low levels of IL-4 transcripts were detected in the CNS of TMEV-infected SJL and B6 mice during early acute disease (70). IL-4 is a growth factor for CD4 and CD8 T

lymphocytes, B lymphocytes, and macrophages, although it appears that is not as potent a growth factor as IL-2 (1, 161, 211, 346). IL-4 induces the expression of MHC class I and II molecules on cells of the monocyte/macrophage lineage. IL-4, TGF-β, and IL-10 are potent inhibitors of monocyte/macrophage activation (2, 73, 502). IL-4 inhibits IL-2-induced CTL activity (433, 453, 487) and inhibits the production of IL-1, IL-6, TNF-α, and IL-12 by STAT6-dependent and -independent mechanisms (151, 238, 459). We have found high levels of IL-4 transcripts in the spinal cord of TMEV-infected SJL mice with late chronic demyelinating disease (70). IL-4 produced during late chronic demyelinating disease may be critical in downregulating the proinflammatory cytokine response in the CNS of these mice and in contributing to the deactivation of cells of the monocyte/macrophage lineage. It may inhibit the generation of antiviral CTL and, in this way, may contribute to the perpetuation of viral persistence during late chronic demyelinating disease. However, Sato et al. (408) reported that high levels of IL-4 were not required for the development of late chronic demyelinating disease in TMEV-infected mice.

Cytokines in CNS Lesions of Patients with MS

Both inflammatory and anti-inflammatory cytokines have been identified in the brain plaques of patients with MS (reviewed in reference 51). TNF-α (158) is associated with astrocytes and macrophages. IFN-α, IFN-β, and IFN-γ have been found at the sites of chronic lesions (468). IFN-γ has been found on astrocytes juxtaposed to active, chronic lesions, while IFN-α has been detected on macrophages (468). In addition to TNF-α and interferons, IL-1, IL-2, IL-4, IL-10, TGF-β (64) were also detected in active chronic lesions. IL-1 was often found in the demyelinating lesions in the hypothalamus (164). IL-2 and IL-4 were also found in acute lesions (505). IL-4 and IL-10 were strongly associated with astrocytes in active, chronic lesions (165), while macrophages expressed IL-10. De Groot et al (92) have found increased expression of TGF-β1, TGF-β2, and TGF-β3 on perivascular and parenchymal macrophages and astrocytes in active demyelinating lesions. TNF-α is capable of inducing oligodendrocyte proliferation and remyelination (19, 429). In one study where patient biopsy specimens were analyzed at 33 and 76 days after the onset of acute MS (42), it was shown that high TNF-α and inducible nitric oxide synthase (iNOS) expression was associated with demyelination (42).

The cytokine pattern found in MS lesions in the CNS is complex and includes both proinflammatory and anti-inflammatory cytokines. It is not different from that found in the CNS of TMEV-infected SJL mice with late chronic demyelinating disease.

ROLE OF NITRIC OXIDE IN TMEV-INDUCED DISEASE AND IN MULTIPLE SCLEROSIS LESIONS

Nitric oxide (NO) produced by iNOS (type II NOS) is a multifunctional molecule which plays an important role in the regulation of immune defenses (48, 312, 319). NO is a highly reactive molecule produced by the conversion of L-arginine to L-citrullin (209, 273, 311). In contrast to constitutively produced neuronal NO (produced by type I NOS) and endothelial NO (produced by type III NOS), NO generated by iNOS is the primary form of NO in acute and chronic inflammation and can be produced in large amounts for prolonged periods (311, 319). Expression of iNOS is activated by LPS and a number of cytokines, such as TNF-α, IFN-γ, IL-1 (272, 312). NO is produced during innate and adaptive phases of the immune response. During an innate immune response, it contributes to the killing of intracellular bacteria and parasites (45, 68, 133, 507). However, it may also have regulatory functions on the cells of the immune system by controlling the level of apoptosis of autoreactive T cells, by controlling the Th1-Th2 balance, by affecting the function of APC, by regulating the expression of the adhesion molecules, and by immunosuppressing the immune responses through inhibition of T-cell proliferation (210, 305, 434). Cytokine-mediated transcriptional induction of iNOS gene in cells of human origin involves the AP-1 and the NF-κB binding sites (272). In the CNS, cytokine-induced iNOS is expressed mainly by microglia/macrophages and astrocytes (156, 166, 521). It has been suggested that excessive production and accumulation of NO by these cells may contribute to neurodegeneration in a number of inflammatory conditions of the CNS (91, 287, 296). NO by itself serves as an antioxidant by scavenging oxygen free radicals. However NO may directly react with proteins, in particular with heme-containing enzymes including guanylate cyclase, cytochrome 450, hemoglobin, and COX-2. NO affects enzymes necessary for mitochondrial energy production and DNA replication and repair (448, 499). Reaction of NO with superoxide results in the formation of highly reactive intermediates such as peroxynitrite, a molecule which can induce lipid peroxidation, DNA strand breaks, and nitrosylation of tyrosine in proteins (26, 254). Peroxidation of membranes and swollen oligodendrocytes have been detected in the CNS of patients with MS (166). NO produced by microglia may also mediate TNF-α neurotoxicity toward oligodendrocytes (423). Because of these characteristics of NO (immunoregulatory and potential cytotoxic), many investigators examined the role of NO in inflammatory demyelinating diseases of the CNS, including virus-induced encephalitis and MS.

We (327, 334) and others (175, 256, 396) have examined whether NO plays a role in the pathogenesis of inflammatory demyelinating disease induced by TMEV. We have demonstrated high levels of iNOS transcripts in the brain and spinal cords of TMEV DA-infected susceptible (SJL) and TMEV-infected resistant (B6) strains of mice during early acute disease, at 6 and 10 days p.i. (327). Immunohistochemical staining revealed that reactive astroglia and cells of the macrophage/microglia lineage expressed iNOS in the CNS (327). No iNOS transcripts or protein were detected in naive and mock-infected animals. In contrast, we have not detected any iNOS transcripts or protein on day 67 p.i. (advanced stage of demyelinating disease) and on day 180 p.i. (terminal stage of disease associated with hind leg paralysis and incontinence) in the CNS of B6 and SJL mice, although infected SJL mice showed severe progressive demyelination and inflammatory lesions.

Foamy, myelin-laden macrophages and rod-shaped microglia were always iNOS negative. However, on days 39 to 42 p.i., weak iNOS staining was detected in reactive astrocytes surrounding areas of necrotizing inflammation in the midbrain and in cells of the monocyte/macrophage lineage in leptomen-

ingeal and white matter perivascular infiltrates of the spinal cords of TMEV-infected SJL mice. Days 39 to 42 p.i. correspond to the early phase of demyelinating disease in TMEV DA-infected SJL mice. Although we did not detect any iNOS transcripts or protein during the advanced and terminal stage of demyelinating disease induced by TMEV in SJL mice, it is possible to envision that NO produced during early gray matter disease and at early stage of demyelinating disease (days 39 to 42 p.i.) could contribute to myelin and oligodendrocyte damage in the CNS of infected mice by the production of peroxynitrite and formation of nitrotyrosine, although this has not been examined. Treatment of TMEV DA-infected mice with aminoguanidine (AG), an iNOS inhibitor, initiated on day 7, 14, or 28 p.i. for 21 days, resulted in a reduction of inflammation, demyelination, and necrosis (396). This protective effect of AG was most effective when TMEV-infected mice received the iNOS inhibitor between days 14 and 32 p.i. However, when TMEV-infected mice received AG between days 66 and 87 p.i., no effect on demyelination was observed and inflammation was only minimally reduced. These results support our conclusion that NO produced during early acute disease and at the beginning of demyelinating disease may be sufficient to initiate myelin damage. The authors suggested that the protective effect of AG on inflammation and demyelination is probably due to the inhibition of apoptosis of oligodendrocytes. In contrast, AG treatment of SJL mice infected with another strain of TMEV, BeAn, during the induction phase of disease (between days 1 and 12 p.i.) did not affect the level of demyelination and inflammation or the clinical score (175). However, treatment of BeAn-infected mice at the effector phase (between days 15 and 26 p.i.) significantly reduced the clinical course of inflammatory demyelinating disease. In both experiments, mice were evaluated on day 75 p.i. The kinetics of the disease induced by TMEV BeAn is substantially different from that induced by the DA strain (Table 1). The encephalitic stage of disease is heavily attenuated in BeAn-infected mice, and iNOS positive cells become detectable at a rather low level at 15 days p.i. and reach maximum at 60 days p.i. Furthermore, the level of iNOS-positive cells appears to parallel the progression of inflammatory demyelinating disease in TMEV BeAn-infected mice. Interestingly, iNOS was detected at low levels in monocytes/macrophages in leptomeninges and perivascular spaces but not in astrocytes (175).

The results of these experiments suggest that the effect of NO on inflammatory demyelinating disease induced by the DA and BeAn strains of TMEV depends on the kinetics of the disease, the time of appearance of inflammatory cells, and the types of cells which express iNOS. Whether NO contributes to demyelination and inflammation in TMEV-infected animals by exerting a direct cytotoxic effect on oligodendrocytes or by affecting apoptosis and/or infiltration of mononuclear cells (at least in animals infected with the BeAn strain) is not clear. However, NO cannot be the only factor contributing to demyelinating disease, because apparently similar levels of iNOS-induced NO were observed in TMEV-infected resistant B6 mice. Interestingly, it has been demonstrated that NO could play a protective role in viral encephalitis (61, 413, 479), could contribute to pathogenesis (35, 129, 419), or could play a minimal role in viral infection (25, 224, 503).

Reactive oxygen and reactive nitrogen species, as well as a number of proinflammatory cytokines, including IL-1, IFN-γ, TNF-α, and IL-12, have been detected in active MS lesions. NO and its reactive derivative peroxynitrite may be implicated playing a role in the pathogenesis of MS. Recent studies indicate that NO and peroxynitrite may be cytotoxic to neurons and oligodendrocytes and that NO may contribute to the destruction of axons (131, 256). The initial studies that examined the expression of NO in MS lesions by NADPH diaphorase staining indicated that astrocytes were the major cell type which produced NO (44, 334). In contrast, monocytes/macrophages, but not astrocytes, have been indicated as cells expressing iNOS by two other groups (23, 93). We have demonstrated expression of iNOS in both acute MS and diffuse sclerosis-type lesions (a subacute form of demyelinating disease in children) but not in chronic MS lesions (334). In acute MS lesions, iNOS was expressed in both monocytes/macrophages and reactive astrocytes, while in diffuse sclerosis-type lesions, it was expressed only in a subpopulation of reactive astrocytes (334).

Staining for nitrotyrosine of acute MS lesions revealed a codistribution of iNOS and nitrotyrosine in cells of the monocyte/macrophage lineage. In diffuse sclerosis-type lesions, nitrotyrosine staining was present in hypertrophic astrocytes. Again, nitrotyrosine staining was absent in chronic MS lesions (334). These results have been confirmed using in situ hybridization and immunohistochemistry, which demonstrated again the presence of iNOS and nitrotyrosine in active lesions but not in chronic lesions (256). The authors detected intense nitrotyrosine staining within the parenchyma, with distribution patterns associated with cell membranes and/or myelin membrane, and in close proximity to vessels, and they suggested that damage to endothelium by peroxynitrite could contribute to the damage of the blood-brain barrier (256). One of the known effects of tyrosine nitration on protein function is inhibition of phosphorylation affecting the number of cellular functions including signal transduction (276). It is very likely that tyrosine-positive cells are eliminated from chronic MS lesions, as described for EAE (512). It is possible that NO plays a dual role in the inflamed CNS: a regulatory, immunosuppressive role by induction of apoptosis of infiltrating T cells and macrophages (at least in TMEV-induced disease) and a role involving cytotoxicity to myelin by formation of peroxynitrite. The latter role would depend on availability of superoxide and other reactive oxygen species.

APOPTOSIS OF T CELLS IN THE CENTRAL NERVOUS SYSTEM OF TMEV-INFECTED MICE AND IN PATIENTS WITH MULTIPLE SCLEROSIS

Apoptosis is essential for maintaining homeostasis of the immune system and plays an important role in the regulation of virus-induced T-cell responses, immune suppression, appearance of memory T cells, and survival of target cells (10, 17, 57, 144, 236, 306, 484). Apoptosis is also an important mechanism of elimination of autoreactive pathogenic lymphocytes, which otherwise could lead to tissue destruction and to autoimmune disease (523).

Apoptosis of Infiltrating T Cells in the CNS of TMEV-Infected Mice

We have examined whether mononuclear cells infiltrating the CNS of TMEV-infected mice are regulated by apoptotic mechanisms (326). This is an important question since these cells play an important role in the clearance of the virus but also may play a important role in the induction and propagation of demyelinating disease.

As discussed earlier in this review, during early acute disease the virus replicates in the gray matter of infected mice. At 3 days p.i., the titer of the virus reaches maximum in both strains of mice (10^6 to 10^7 PFU/g of CNS), and as early as 10 days p.i., the virus is completely eliminated from the CNS of B6 mice. In contrast, the virus is never completely cleared from TMEV-infected SJL mice, although at 10 days p.i. its titer has been significantly reduced (5×10^2 PFU/g of CNS) (Fig. 1). During the first 3 days p.i., replication of the virus is presumably under the control of an innate immune response. At 3 days p.i., inflammatory infiltrates consisting of $CD4^+$ and $CD8^+$ T cells, monocytes/macrophages, and a few B cells appear predominantly in the regions of the basal ganglia and thalamus of TMEV-infected SJL and B6 mice. These infiltrates increase in cell number through days 7 to 8 p.i. and, by day 20 p.i., are completely eliminated from the CNS of TMEV-infected SJL and B6 mice (243).

To determine whether mononuclear cells infiltrating the CNS of TMEV-infected mice during early acute disease undergo apoptosis, we carried out immunohistochemical staining of brain tissue sections of these mice by using the terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling TUNEL assay (326). We have demonstrated that significant numbers of infiltrating cells are undergoing apoptosis at 8 and 10 days p.i. (326). TUNEL-positive cells were located predominantly in perivascular spaces but were also present in the intervening neuropil. A number of TUNEL-positive neurons or TUNEL-positive macrophages were also detected in areas of active encephalitis in SJL and B6 mice. However, double staining for TUNEL followed by immunohistochemical staining for CD3 revealed that predominantly T cells are undergoing apoptosis in the CNS of TMEV-infected mice. In order to quantitate the T cells undergoing apoptosis during early acute disease, we isolated mononuclear cells infiltrating the CNS of TMEV-infected mice and employed a flow cytometry-based TUNEL assay combined with staining for CD3 (326). Using this method, we established that at the peak of early acute disease (6 to 7 days p.i.), approximately 20 to 30% of $CD3^+$ cells were undergoing apoptosis. In contrast to T cells isolated from the spleens of TMEV-infected mice, T cells infiltrating the CNS of infected mice expressed high levels of Fas and FasL but negligible levels of Bcl-2 (approximately 1%). These results suggest that T cells infiltrating the CNS of TMEV-infected mice during early acute disease are eliminated from the CNS through activation-induced cell death (AICD). This process allows for the clearance of the virus (although the clearance is not complete in SJL mice). Activated T cells produce a number of potentially hazardous molecules, including TNF-α and IL-1, and may contribute to the production of free radicals. The elimination of these molecules is essential to maintain homeostasis in the CNS (6, 8, 279). AICD is mediated by Fas/FasL and/or TNFRI (53, 101, 445, 520). Fas and TNFRI have a common death domain (53, 101, 208, 236, 403, 414, 445, 520). The key components of the death-signaling complex are FADD (an adaptor protein) and the proenzyme form of caspase-8. The aggregation of FADD and caspase-8 results in proteolytic release of the active enzyme. Active caspase-8 initiates a chain of lethal events involving activation of other caspases. This pathway could be blocked by FLIP, a cellular gene product expressed in lymphocytes, which inhibits the enzymatic function of caspase-8.

Thus, infection of the gray matter with TMEV during early acute disease follows a well-recognized pattern. There is an original expansion of T cells infiltrating the CNS in response to viral infection, associated with TCR activation, followed by up-regulation of the Fas/FasL system and resolution of the inflammatory response through AICD after viral clearance. Presumably some of the infiltrating T cells will survive as memory cells.

In contrast, during late chronic demyelinating disease that is associated with heavy meningeal, perivascular, and parenchymal infiltration by $CD4^+$ $CD8^+$ cells and cells of the monocyte/macrophage lineage, very few $CD3^+$ cells are undergoing apoptosis as determined by ex vivo TUNEL combined with anti-CD3 staining (326). At 6 months p.i., fewer than 2% of $CD3^+$ cells were undergoing apoptosis. However, expression of Fas and FasL remained high during late chronic demyelinating disease. The negligible levels of apoptosis of $CD3^+$ cells expressing Fas and FasL in the CNS of infected mice could be attributed to the high levels of expression of Bcl-2 by T cells. At approximately 5 months p.i., about 30% of all $CD3^+$ cells infiltrating the CNS of TMEV-infected mice expressed Bcl-2 (326). It appears that the lack of apoptosis of T cells during late chronic demyelinating disease leads to the accumulation of these T cells in the CNS. Because the CNS is a semiprivileged organ and can tolerate inflammation only for short periods, persistent inflammation with activated $Fas^+/FasL^+$ T cells probably plays a role in the induction and/or propagation of demyelinating disease. The mechanism of upregulation of Bcl-2 in these T cells may be of utmost importance for understanding the pathogenesis of late chronic demyelinating disease. Bcl-2 may be upregulated by a number of cytokines, including IL-2 and IL-4, both of which have been detected in the CNS of TMEV-infected mice during late chronic demyelinating disease. Bcl-2 is found on both naive and memory T cells (both CD4 and CD8) (326). A comparison of the proportions of $CD3^+$ TUNEL-positive cells to those of $CD3^+$ $Bcl\text{-}2^+$ cells in the CNS of TMEV-infected SJL mice during early acute disease and late chronic demyelinating disease is shown in Fig. 2.

In other experiments, we have phenotypically characterized T cells infiltrating the CNS of TMEV-infected mice. During early acute disease, most infiltrating T cells were $CD44^{hi}$ $CD62L^-$, suggesting the presence of activated T cells. Naive T cells ($CD44^{low}$ $CD62L^{hi}$) were practically undetectable. In contrast, during late chronic demyelinating disease, most infiltrating T cells exhibited characteristics of the effector/memory cells ($CD44^{hi}$ $CD62L^{low}$) (B. E. Hoffman, J. Robert Chang, C. D. Platsoucas, and E. L. Oleszak, unpublished data). Therefore, at least a proportion of T cells present in the CNS of TMEV-infected mice expressing Bcl-2 may represent memory

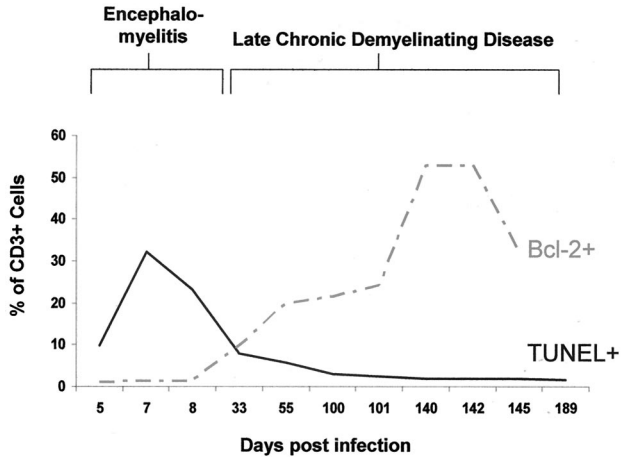

FIG. 2. Kinetic study of the proportions of $CD3^+$ TUNEL-positive cells and of $CD3^+$ $Bcl\text{-}2^+$ cells in the CNS of TMEV-infected SJL mice during early acute disease and late chronic demyelinating disease. During early acute disease, high proportions of $CD3^+$ TUNEL-positive cells and minimal proportions of $CD3^+$ $Bcl\text{-}2^+$ cells were observed in the CNS of these mice. However, during late chronic demyelinating disease, the proportions of $CD3^+$ TUNEL-positive cells were minimal and those of $CD3^+$ $Bcl\text{-}2^+$ cells were high in the CNS of TMEV-infected SJL mice. Data from reference 326.

cells. It is not known whether these memory cells are specific for a viral or a host (self) epitope.

A number of investigators have examined apoptosis induced by TMEV in tissue culture or in ex vivo systems. Anderson et al reported that TMEV induces rapid necrosis and delayed apoptosis in myelinated mouse cerebellar explants (16). Jelachich and Lipton found that TMEV (both the GDVII and BeAn strains) induces apoptosis in restrictive but not in permissive cells (184) and in macrophages (182). They extended their studies to activated macrophages and defined the mechanisms by which these cells were dying after BeAn infection (183). They concluded that two independent mechanisms were involved, an intrinsic pathway which required virus replication and an extrinsic pathway signaling through TRAIL receptors (183). Studies by Palma et al. suggested that $CD4^+$ T cells may mediate the apoptosis of astrocytes in TMEV-infected mice and perhaps contribute to the breaching of the blood-brain barrier (344). Recently, Ohara et al. (321) reported that TMEV could infect a microglial cell line but did not induce apoptosis, a process which could lead to viral persistence.

Apoptosis of T Cells in Patients with MS

Several investigators suggested that MS may be an autoimmune disease with a genetically determined impairment of apoptosis of T cells. However, this hypothesis was based on certain animal models of inflammatory demyelinating disease such as EAE. Studies of the apoptosis of T cells infiltrating brain lesions of patients with MS are limited. Dowling et al. (108) demonstrated that remarkably few apoptotic T cells could be detected in the brains of patients with massive perivascular infiltrates. In separate studies, Zettl et al. (517) analyzed the presence of Bcl-2-positive T cells in the CNS of patients with remitting-relapsing disease and with the primary progressive course of the disease. They concluded that the percentage of Bcl-2-positive cells depended on the activity of the lesion and on the course of the disease. In patients with remitting-relapsing MS, a rather low percentage of T cells in active lesions expressed Bcl-2, suggesting that these infiltrating T cells may undergo apoptosis, a process which could lead to resolution of inflammation until the next relapse. In contrast, a much higher proportion of T cells expressed Bcl-2 in inflammatory lesions in the brains of patients with primary progressive MS, perhaps contributing to the chronic nature of the disease and the lack of remission. These reports, describing an inverse relationship between the expression of Bcl-2 and apoptosis in the primary progressive type of MS, are strikingly similar to late chronic demyelinating disease induced by TMEV. In TMEV-infected mice, a paucity of apoptosis was also associated with expression of Bcl-2 and resulted in the accumulation of T cells in the CNS.

Several reports published recently suggested impaired apoptotic deletion of MBP-reactive T cells in the peripheral blood of patients with MS. Zang et al. (514) demonstrated that a significant proportion of T cells in the peripheral blood of patients with MS, which are specific for MBP, are sensitive to FasL-mediated apoptosis but are not deleted in vivo. Defective apoptotic deletion of mitogen-stimulated T lymphocytes in the peripheral blood of patients with MS has been reported by several groups of investigators. This impairment of apoptosis has been attributed to a high expression of FLIP (a potent inhibitor of death receptor signaling transduction) in activated lymphocytes from patients with clinically active MS (425). In contrast, the expression of Bcl-2 and Fas was relatively similar between MS patients with active or stable disease and was similar between the MS groups and controls consisting of T cells from the peripheral blood of patients with inflammatory neurological disorders and noninflammatory neurological disorders (430). However, the same group of investigators extended their studies to other members of the Bcl-2 family and reached the conclusion that there was a significant reduction in the expression ratios of proapoptotic to antiapoptotic members of the Bcl-2 family in lymphocytes derived from peripheral blood and CSF of patients with MS compared to corresponding ratios in control groups or healthy individuals (430). Elevated levels of Bcl-XL (a survival factor within the Bcl-2 family) but not of Bcl-2 on T cells from the peripheral blood of patients with MS or from the peripheral blood of normal donors have been reported (489). Finally, Sharief and Semra (432) reported upregulation of certain IAP (inhibitors of apoptosis proteins), namely, IAP-1, IAP-2, and XIAP, in T lymphocytes in the peripheral blood of patients with active but not stable MS. IAP are direct inhibitors of apoptosis by blocking the downstream effector caspase-3 and -7 and procaspase-9 (98).

In summary, T lymphocytes from the peripheral blood of patients with MS are less susceptible to AICD through numerous impairments of the apoptotic pathways. Although no differences in Bcl-2 expression on T cells from the peripheral blood of patients with MS and those from appropriate controls have been reported, studies with TMEV-infected mice suggest that this antiapoptotic molecule may be expressed by T lymphocytes infiltrating the targeted organ (the CNS).

TMEV AND ANTIBODY RESPONSES

Antiviral antibodies are routinely found in the CNS of TMEV-infected SJL mice (249, 372). Given that much higher antibody titer can be found in the CNS than in the serum (249), a local antibody response to TMEV seems most likely. Further evidence for local antibody responses in the CNS was shown by anodal distribution of anti-TMEV antibody in the CSF, but not in the serum, using isoelectric focusing followed by the immunoblotting antigen overlay technique (394). Characterization of the anti-TMEV antibodies demonstrated that neutralizing antibodies predominantly targeted the VP1 (78, 169, 317, 508) and the VP2 (78, 508) capsid proteins.

Of particular interest is the linear epitope $VP1_{262–276}$ (named A1Cb) (78, 205, 508). In BeAn-infected SJL mice, A1Cb was the major target of the B-cell response, whereas in resistant BALB/c and C57BL/6 strains of mice, multiple epitopes were found (169). Antibodies recognizing A1Cb were greatly efficient in neutralizing TMEV in vitro (169), most probably due to A1Cb being in such close proximity to the viral receptor binding site (169, 269).

In vivo, A1Cb becomes available as an epitope through the trypsin cleavage of TMEV viral capsid proteins (317, 322). In contrast, the major epitopes for neutralization in resistant BALB/c mice are the trypsin-cleavage independent epitopes A1A ($VP1_{13–27}$) and A2A ($VP2_{2–14}$) (205). Interestingly, trypsin cleavage of the VP1 capsid protein renders TMEV more infectious (322). Therefore, we see yet another difference in susceptibility to persistent TMEV infection in that SJL mice are able to develop an efficient antibody response only upon trypsin cleavage of the viral capsid proteins. This suggests that the inability to clear the primary TMEV infection (due to an inefficient CTL response [99]) may lead to the more infectious form of TMEV. It is even conceivable that the trypsin cleavage would contribute to the spread of the virus from the gray matter to the white matter, where the virus establishes a persistent infection.

It has been noted that high A1Cb-specific antibody responses were seen in clinically symptomatic SJL mice (508), suggesting the emergence of active demyelination. Those A1Cb-specific antibodies present in clinically symptomatic mice were mostly of the IgG2a subclass (169), in agreement with the notion that inflammation of the CNS during active demyelination may be attributed, in large part, to the Th1 response. This is further confirmed by studies showing that skewing the immune response from Th1 to Th2 by using ethylene carbodiimide-treated splenocytes coupled to UV-inactivated TMEV, either before or after infection with live TMEV, leads to the amelioration of the late chronic demyelinating disease (194, 195, 351).

Immunizing SJL mice with synthetic A1Cb, A1A ($VP1_{12–25}$), and A1B ($VP1_{146–160}$) peptides coupled to keyhole limpet hemocyanin significantly decreased the incidence of TMEV-associated late chronic demyelinating disease (508). In contrast, in TMEV-infected SJL mice without preimmunization, the onset of clinical symptoms was associated with high levels of antibody responses to A1Cb (508). Therefore, it appears that antibody neutralization of TMEV to A1Cb is insufficient. Furthermore, the antibody response alone is inefficient in completely preventing TMEV pathogenesis, and viral clearance by CTL responses plays a more significant role in TMEV pathogenesis.

Although antibody does not contribute greatly to viral clearance, SJL mice depleted of B- cells by treatment with goat anti-μ (anti-μ IgG) (381) had more severe demyelinating lesions at 35 days p.i. Strains of mice resistant (C57BL/10, C57BL/6, and B10.D2) to TMEV-induced late chronic demyelinating disease did not develop demyelinating disease after treatment with goat anti-μ (217, 381). When SJL mice were depleted of complement by treatment with cobra venom factor, the animals developed, as in the B-cell depletion study, a more severe demyelinating disease (384). These findings suggest that humoral responses in TMEV-induced late chronic demyelinating disease in susceptible mice contribute more to ameliorating or delaying the onset of demyelination than to clearing the virus, whereas in resistant strains of mice, efficacious CTL responses during the early stages of TMEV pathogenesis lead to viral clearance and prevent the development of late chronic demyelinating disease.

In addition to virus-specific antibody responses, anti-MBP antibody can be found in the sera of TMEV-infected mice with demyelinating disease (372), which is attributable to the presence of MBP in the CSF of chronically infected mice. Interestingly, anti-MBP responses are seen only in the sera while TMEV-specific antibody responses are seen in the CSF (372). Further studies of MBP-specific antibodies gave rise to the identification of MAbs SCH94.03, SCH94.32 (292), and SCH79.08 (20), of the IgM class, which promoted CNS remyelination. Of note, MAbs SCH94.03 and SCH94.32 were found to react with glial cell lines originating from rats and mice as well as from humans (292). In addition, the same MAbs were able to interact with neural, fibroblast, epithelial, and lymphocytic cell lines, indicating that the determinants recognized by these MAbs are widely expressed (292).

When MAb SCH94.03 was administered to TMEV-infected SJL mice by passive transfer, there was a significant decrease in the number of T cells infiltrating the CNS, a decrease in the humoral response to TMEV, a two- to threefold increase in viral antigen expression, and the promotion of remyelination at 6 to 8 months p.i. (289). Immunohistochemical labeling demonstrated that MAb SCH94.03 bound oligodendrocytes, myelin sheaths, and axons within demyelinating lesions (167) and MHC class II-positive dendritic cells in lymphoid organs (289). Since MAbs SCH94.03 and SCH79.08 promoted remyelination by binding to different autoantigens on oligodendrocytes, it appears that the binding of MAbs to oligodendrocytes is sufficient to induce remyelination. Although these autoantibodies also decrease inflammation in the CNS, it is not clear whether the triggering of remyelination by them is due to indirect suppression of inflammation (289) or to direct activation of oligodendrocytes to produce myelin (20) or both.

IVIg Treatment of MS

Along the same lines, polyspecific antibody (IVIg) treatment of patients with MS has shown some success (439, 440, 446), possibly by inducing T-cell suppression (9), by decreasing the endocytosis of MBP (443), and by protecting oligodendrocytes from complement injury (442). Like MAbs SCH94.03 and SCH79.08 administration of IVIg to TMEV-infected mice also

increased remyelination (491). However, as in EAE (168) polyclonal human IgM was more effective in inducing remyelination in TMEV-infected mice (491). Furthermore, human IgM capable of binding oligodendrocytes also promoted remyelination at levels similar to that induced by polyclonal human IgM treatment.

IVIg may promote immunosuppression by signaling through the Fcγ receptor. Sutterwala et al. have demonstrated that the ligation of Fc receptor induces IL-10 expression (449). In addition, Fcγ receptor ligation suppressed IL-12 expression (145). Recently, Nakahara et al. further reinforced the use of IVIg treatment by showing that oligodendrocytes produce myelin on Fc receptor ligation (310).

CONCLUDING REMARKS

Although progress has been made, the mechanism(s) of demyelinating disease induced by TMEV or observed in patients with MS has not been determined. The questions that need to be answered include the following.

(i) What is the contribution of the T cells specific for the virus versus the T cells specific for self-neuroantigen(s) in the development of demyelinating disease?

(ii) What is the role of persistent infection in the development of demyelinating disease?

(iii) What is the molecular mechanism of migration of the virus (TMEV) from the gray matter to the white matter and the concomitant infection of glial cells?

(iv) Lessons from the studies of TMEV pathogenesis and epidemiological studies of MS strongly suggest that virus(es) is the initial trigger of MS. Therefore, what is the virus(es) associated with MS, and what role does persistent infection play in the pathogenesis of MS?

(v) Is MS exclusively an autoimmune disease?

(vi) What is the mechanism(s) of axonal injury in TMEV-induced disease and in patients with MS?

(vii) How do NO and reactive oxygen intermediates contribute to myelin and/or oligodendrocyte injury, and does NO play a regulatory role the development of demeyelinating disease?

(viii) What is the molecular mechanism(s) involved in the genetically determined failure of T-cell apoptosis in patients with MS and in TMEV-infected mice during late chronic demyelinating disease?

(ix) Are viral infections of the CNS before puberty responsible for the development of clinically apparent MS in adulthood?

Addressing these questions will enhance the development of new molecular approaches to the management of demyelinating diseases of the CNS.

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