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Two Models of Multiple Sclerosis: Experimental Allergic Encephalomyelitis (EAE) and Theiler's Murine Encephalomyelitis Virus (TMEV) Infection. A Pathological and Immunological Comparison

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ABSTRACT Theiler's murine encephalomyelitis virus (TMEV) infection and experimental allergic encephalomyelitis (EAE) are considered among the best models of human multiple sclerosis (MS). In both models, clinical disease is characterized by paralysis, while pathological changes consist of inflammatory demyelination. In both models there is a genetic influence on susceptibility/resistance to the development of disease. This has been thoroughly studied in TMEV infection, and it has been found to depend on both major histocompatibility complex (MHC) and non-MHC genes. At least four genes have been so far identified. Because of this genetic influence, some strains of mice are more susceptible to both clinical and pathological changes than others, and susceptibility appears to best correlate with the ability of a certain murine strain to develop a delayed-type hypersensitivity (DTH) response to viral antigens. We have also observed that even among mice which are equally susceptible clinically, striking differences may be seen under pathological examination. These consist of different gradients of severity of inflammation, particularly in regards to the macrophage component. There is an inverse relationship between the number of macrophages, and their length of stay in the CNS, and the ability of mice to remyelinate their lesions. The most severe lesions are in SJL/J mice, and remyelination in this strain is extremely poor. The least severe lesions in terms of macrophage invasion are in strains such as NZW and RIIIS/J, and these are able to remyelinate lesions very successfully. Murine chronic relapsing EAE (CR-EAE) shows pathological changes in many ways similar to those in TMEV-infected SJL/J mice, although less severe in terms of degrees of macrophage infiltration and tissue destruction. Mice with CR-EAE have a correspondingly limited ability to remyelinate their lesions. In both models the pathology appears to be mediated through a DTH response. However, while in EAE the DTH response is clearly against neuroantigens, the response in TMEV infection is against the virus itself. The end result in both models would be that of myelin destruction through a lymphotoxin-cytokine-mediated mechanism. The importance of the DTH response in both models is well illustrated by the effects of tolerance induction in EAE and TMEV infection to neuroantigens and virus, respectively. These are important models of human MS, since the current hypothesis is that a viral infection early in life, on the appropriate genetic background, may trigger a secondary misdirected immune response which could be directed either against myelin antigens and/or possible persistent virus(es).

INTRODUCTION

The causes and mechanisms underlying the development of demyelination in multiple sclerosis (MS) are still unknown. During the past several decades, studies in both humans and experimental animal models have been conducted in order to clarify the relationships between a presumed genetically determined immune regulatory dysfunction in MS patients and possible environmental factors. Epidemiological data, particularly those regarding the increased frequency of MS in the Faroe islands after the invasion of British troops during World War II (Kurtzke and Hyllested, 1979), have suggested that a possible relation may exist between a viral infection during childhood and early adolescence and the subsequent development of demyelination in individuals with the appropriate genetic background (Poser, 1993). Because of these considerations, in the last couple of decades a number of animal models in which a viral infection triggers a chronic process of

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demyelination have been established. Viral models such as coronavirus infection in mice and rats, herpes simplex virus types I and II infection in mice, canine dystemper in dogs, and visna infection in sheep, among others, are all excellent models, and they have been recently reviewed (Dal Canto, 1990). In this paper we shall focus on two models we have been studying for the last several years, Theiler's murine encephalomyelitis virus (TMEV)–induced demyelinating disease and murine chronic relapsing experimental allergic encephalomyelitis (CR-EAE). These models have similarities as well as differences which we shall try to illustrate. Both models have interesting features, both pathologically and immunologically, that make them among the best models for human MS. We shall develop them in parallel, starting with the TMEV model, and then interweave them as we go along in order to best illustrate some of the most important pathogenetic differences.

THEILER'S MURINE ENCEPHALOMYELITIS VIRUS INFECTION

Theiler's virus is a picornavirus, recently classified among the subgroup of cardioviruses (Pevear et al., 1987), and a natural pathogen for mice (Theiler, 1937). In the wild, it produces an infection of the enteric tract of the mouse and, occasionally, a paralytic disease as well. It is one of the first viruses to be recognized as able to produce a chronic infection, a feature that makes this model a very important tool for investigating mechanisms of viral persistence (Lipton, 1975). There are two important subgroups of TMEV. One, consisting of GDVII and FA viruses, is highly virulent and produces a fulminant encephalitis resulting in death in less than a week in most animals. The second, called TO group, from Theiler's original, includes the Daniels (DA) and the BeAn 8386 strains, which produce a chronic persistent infection in the CNS, resulting in inflammatory demyelination (Lipton, 1980). The DA strain also produces an initial, self-limited, distinct phase of grey matter involvement (Dal Canto and Lipton, 1975; Lipton, 1975), while the BeAn strain involves grey matter in minimal degree. While our initial studies were done using the DA strain, in the last several years we have preferred the use of the BeAn strain, which is tissue culture adapted.

Clinical Presentation

Animals are infected at about 6–8 weeks of age through an intracerebral injection of 2.9×10^6 plaque-forming units (PFU) of virus, while controls are similarly injected with DMEM under methoxyflurane anesthesia. In the most susceptible strains clinical signs begin to develop at about 5 weeks after infection and slowly progress from a mild waddling gait to frank spastic hind limb paralysis and urinary incontinence after 10–12 weeks of infection. Mice are scored based on the severity of involvement with a numerical system going from zero (unaffected) to four (death).

Susceptibility to Infection

Among inbred mouse strains, there is a spectrum of susceptility to the development of TMEV-induced demyelinating disease after infection (Lipton and Dal Canto, 1979). Strains such as SJL/J, DBA/1, DBA/2, SWR/J, NZW, RIIIS/J, and PL/J are highly susceptible. The AKR, CBA, and C3H strains are intermediately susceptible. The C57BL/6, C57BL/10, C57L, and BALB/c strains are resistant, although some variation has been noted among the BALB/c substrains (Nicholson et al., 1994). Multiple genes are involved, both MHC and non-MHC genes, and the loci found to have predominant effects in one strain combination (e.g., C57BL/6 vs. DBA/2) may differ from those in another (e.g., C57BL/6 vs. SWR). In some F1 hybrids between susceptible and resistant strains, resistance is dominant, while susceptibility is dominant in others (Melvold et al., 1987; Blankenhorn and Stranford, 1992).

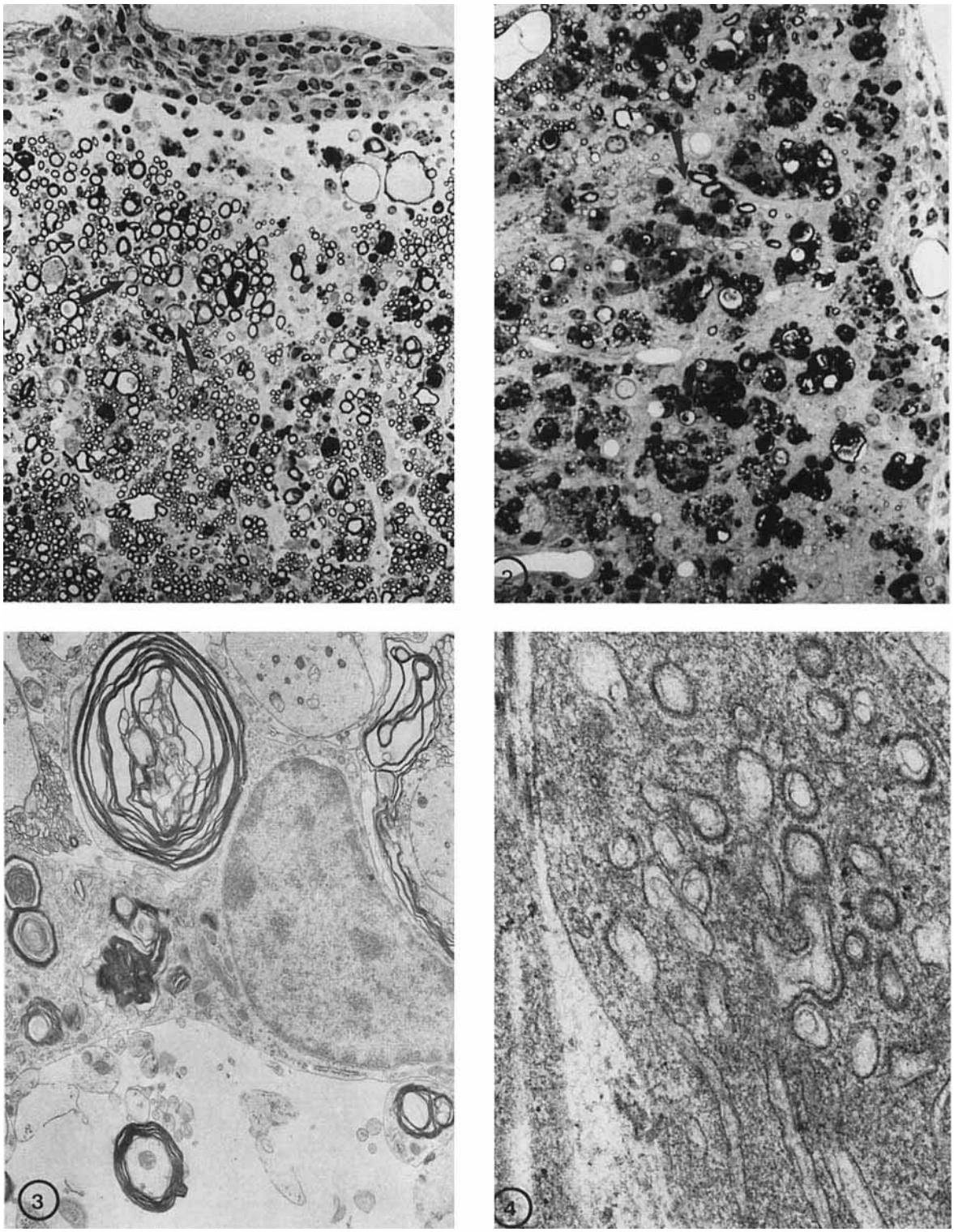
Several specific loci have been identified as being involved in differential susceptibility: the class I MHC locus *H-2D* (Clatch et al., 1985, 1987a; Rodriguez and David 1985; Rodriguez et al., 1986a, 1990; Patick et al., 1990), the *Tmevd-1* locus on chromosome 3 (Melvold et al., 1990), and the *Tmevd-2* locus on chromosome 6 (Melvold et al., 1987). There is also recent evidence for involvement of a fourth locus, determining the differential susceptibility of the SWR and C57BL/6 strains, whose chromosomal location is unknown except that it is not linked with *H-2D, Tmevd-1,* or *Tmevd-2* (S. Nicholson, personal communication). In addition, recent studies of the genetic basis for differences observed in TMEV persistence (which may not equate with development of clinical disease) in different

Fig. 1. Picture of a very early lesion, characterized by leptomeningeal lymphoid infiltrates underneath which early disorganization of white matter is observed. There is increased intercellular space and demyelination of axons (arrows). One large dystrophic axon is seen to the left, and several macrophages with myelin debris are present throughout. One micron thick, Epon-embedded spinal cord section from SJL mouse 4 weeks after BeAn infection, stained with toluidine blue. ×275.

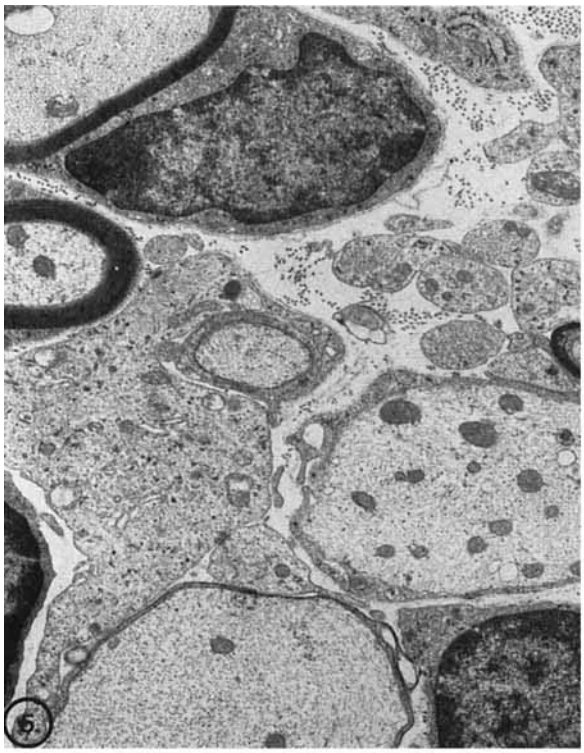
Fig. 2. Typical appearance of spinal cord during the acute to subacute phases of demyelination in BeAn-infected SJL/J mice. Mononuclear inflammatory cells are visible in the leptomeninges (right-hand side of the picture), and a continuous layer of myelin laden macrophages is present in the sub-pial area. Underneath, there is very severe disorganization of the white matter, in which the most striking feature is the presence of very large myelin and lipid-laden macrophages. In the intervening parenchyma, gliosis is observed, and small numbers of naked axons are present (arrow). Acute lesions with this appearance can be observed for many months after infection, since new lesions may form even in the chronic phases of the disease. One micron thick, Epon-embedded section, stained with toluidine blue. ×275.

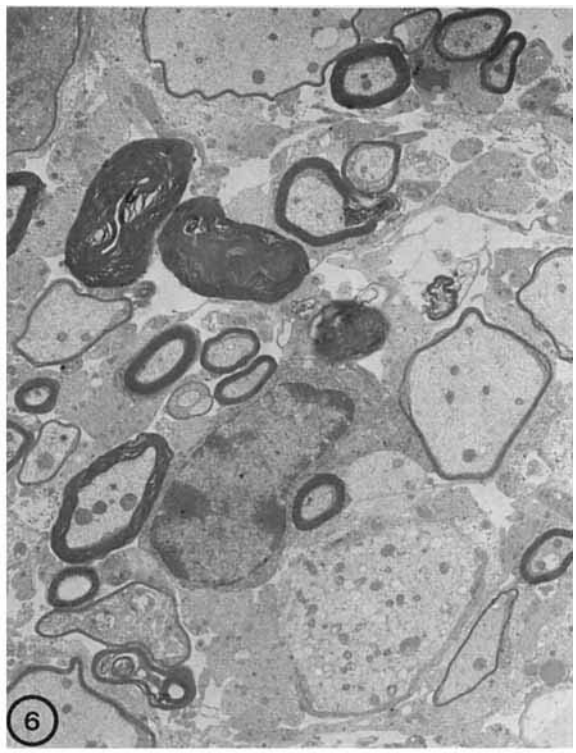
Fig. 3. Ultrastructural appearance of demyelination in spinal cord of an SJL mouse 6 weeks after BeAn infection. In the upper part of the photograph, two axons are observed being demyelinated by the process of vesicular disruption. These axons appear otherwise normal. A macrophage laden with myelin debris is seen in close vicinity of these axons. The macrophage abuts on a greatly expanded extracellular space in which a myelinated axon and some glial processes are immersed. ×28,000.

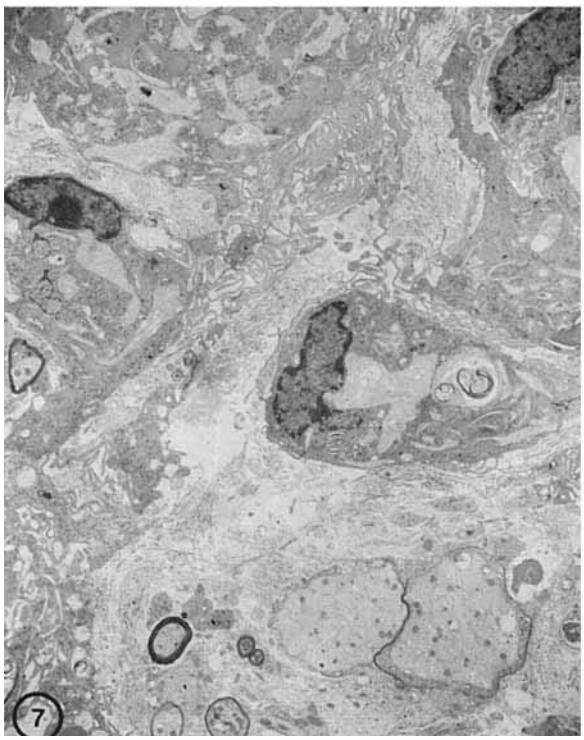
Fig. 4. Numerous coated pits and vesicles are seen in this monocyte. These are strongly reminiscent of profiles described in MS and EAE and interpreted as representing either receptor-mediated phagocytosis or a stage in antigen processing. ×43,000.

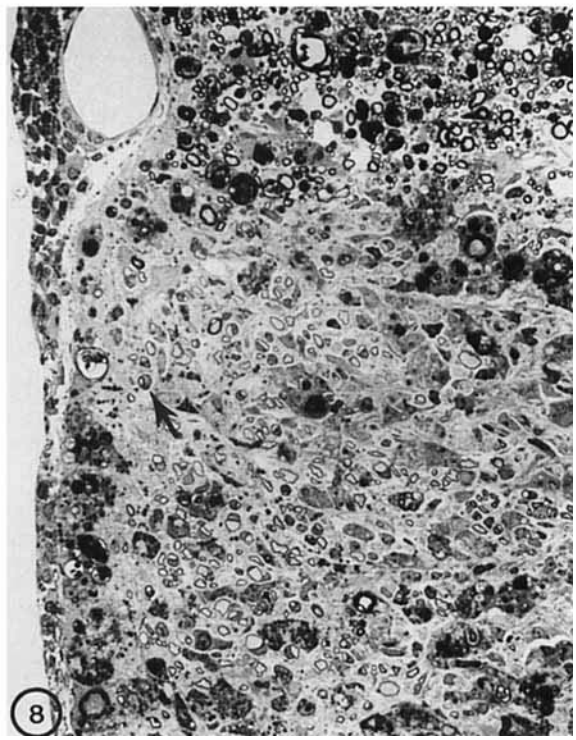


Figs. 1–4.









Figs. 5–8.

strains have implicated the *H-2D* locus as well as non–H-2 loci (Bureau et al., 1992). The reason for difficulties in some of the genetic analyses may be the potential influence of a gene onto another. For example, *H-2D* exerts an overriding effect over *Tmev-2* near the T cell receptor genes (Kappel et al., 1991). In some strain comparisons, sex also contributes to differential susceptibility, in that males have a higher incidence of disease than do females (Kappel et al., 1990).

Pathological Alterations

Some features in the pathology of TMEV infection are common to all susceptible strains examined. Mice generally begin to show the first demyelinating lesions at 3–4 weeks after viral inoculation. In contrast to the DA strain of TMEV, in which a first phase of polio-like disease is clearly observed (Dal Canto and Lipton, 1975), the BeAn virus strain generally produced a very mild initial compromise of the anterior horn neurons in all strains. Neuronal involvement produced by BeAn virus is focal, only appreciable in a minority of sections, and self-limited, since it is almost never observed after 3 weeks of infection. At about that time, on the other hand, white matter involvement begins and continues for the lifetime of the animal, generally with a progressive pace, although in several instances it is possible to appreciate acute lesions, side by side with chronic gliotic areas, months after infection, suggesting a remitting, relapsing pattern as well.

We shall consider pathological alterations only in the strains most susceptible to clinical disease. Interestingly, despite a similar clinical presentation in these animals and similar frequency of infection, pathological alterations can be distinguished into three main categories in different strains. In general, SJL mice present the most destructive lesions, DBA mice present lesions of intermediate severity, and SWR, NZW, RIII/SJ, and some of the BALB/c mice present lesions of least destructive quality. Some points may be made in regard to these different pathological presentations.

SJL/J mice. Lesions in this strain develop quite quickly. They involve both anterior and lateral columns of the spinal cord and are characterized by inflammation and demyelination as well as extensive axonal destruction. They are therefore rather different from the lesions we originally described in SJL/J animals after infection with the DA strain of TMEV. The lesions produced by the DA virus, in fact, were less destructive, and there was much less axonal involvement (Dal Canto and Lipton, 1975).

The initial phases of a lesion are characterized by the presence of meningeal lymphoid infiltrates, around which one begins to see a loosening and disorganization of the white matter architecture. Macrophages are already present, and both axonal and myelin involvement are apparent. Myelin involvement, however, appears to be the most severe so that scattered demyelinated axons can be observed. (Figs. 1, 2). Inflammatory cells aggregate in the subarachnoid space, particularly around meningeal vessels, and extend into the spinal cord by following the perivascular spaces. Eventually, they migrate through the vessel walls and reach the parechyma. These events are very rapid and can generally be observed during the second to third week after infection. Once the inflammatory cells start infiltrating the parenchyma of the spinal cord, severe myelin and considerable axonal compromise begin to appear and quickly worsen thereafter. By 3–4 weeks, lesions may already be coalescent to occupy the majority of the anterior and lateral columns (Fig. 2).

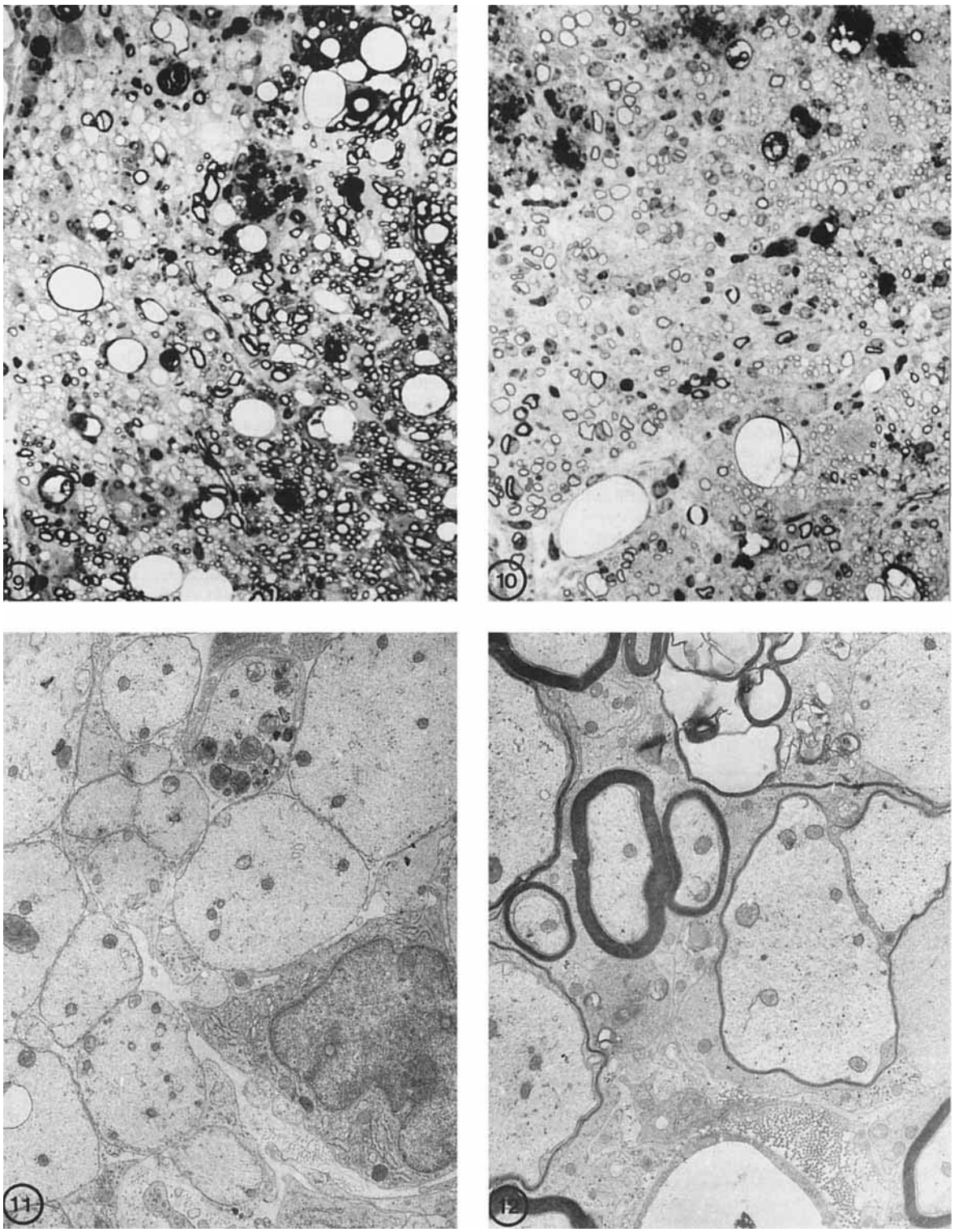
One feature which is particularly evident in SJL/J mice is the presence of large numbers of macrophages which quickly populate the spinal cord columns (Fig. 2). Such macrophages are always present even at the very beginning of demyelinating activity and remain in the parenchyma for extended periods of time. They are generally very large and loaded with lipid droplets as well as more coarse myelin debris. The intervening parenchyma becomes generally hypocellular, with scattered demyelinated axons (Fig. 2). By light microscopy, one has the impression that the majority of the tissue among the numerous macrophages is mainly consistent of reactive glial processes. By electron microscopy, the process of demyelination is shown to be mainly accomplished by the process of vesicular myelin degeneration, always in close relationship with macrophages (Fig. 3). As in MS, and in contrast to the DA model, the process of myelin stripping is rather rare, most myelin being apparently dissolved by vesicular destruction (Prineas, 1985; Raine and Scheinberg, 1988). We and others have interpreted these features as representing myelin destruction by a number of possible soluble effector molecules, liberated by activated lymphocytes and especially by macrophages (Dal Canto et al., 1975; Hofman et al., 1989; Selmaj and

Fig. 5. Two axons have been successfully remyelinated by Schwann cells in the upper left-hand corner of this photograph. The two axons in the lower part of the picture are still demyelinated. The lower axon, however, shows an attempt, albeit unsuccessful, at oligodendroglial remyelination. ×29,000.

Fig. 6. A rare field showing several axons remyelinated by oligodendrocytes. Myelin is abnormally thin. One oligodendrocyte is present in the center of the picture. Several axons show degeneration characterized by either dense axoplasm or a fluffy or vacuolated appearance. This field was taken in close proximity to an area of acute demyelinating activity. ×9,000.

Fig. 7. A representative white matter field in a chronic gliotic plaque. Two demyelinated axons are present in the lower part of the picture, one of which shows a very thin rim of myelin. A few more small axons are present scattered about, but most axons in this area have obviously degenerated and the space has been taken over by both collagen fibers and glial processes. In addition, macrophages with light staining droplets, characteristic of neutral lipids, are seen. SJL mouse, 4 months after infection with BeAn virus. ×9,000.

Fig. 8. A chronic lesion in an SJL/J mouse 10 weeks after BeAn infection. The chronic plaque in the center is flanked by acute activity both above and especially beneath it. The intercellular space is increased in the chronic plaque, and scattered axons appear to have been remyelinated. Closer scrutiny discloses that most of the remyelination has been accomplished by Schwann cells, as evidenced by the presence of their nuclei in close apposition to the respective axons (arrow). Macrophages are present in great number in the fresh lesions, particularly large in the lesion at the lower part of the figure. This photograph illustrates the recurrent nature of the disease in this model. One micron thick, Epon-embedded section, stained with toluidine blue. ×275.



Figs. 9–12.

Raine, 1988). It is of interest that frequent coated pits and vesicles were observed in monocytic cells (Fig. 4), closely recalling a process described in MS and EAE (Epstein et al. 1983; Moore and Raine, 1988; Prineas, 1985). These profiles have been variously interpreted either as the structural representation of receptor-mediated phagocytosis and/or a stage in antigen processing. The extracellular space becomes quite expanded, and axons and other structures appear as floating in it. There is very little remyelinating activity in these spinal cords. When present, it is generally carried out by invading Schwann cells (Fig. 5), although, occasionally, remyelination by oligodendrocytes can be seen (Fig. 6). This is another phenomenon paralleling the situation in human MS, where remyelination and demyelination may take place side by side (Prineas, 1985). In the chronic stage of the disease, the white matter may be severely disorganized, with numerous intersecting glial processes, bundles of collagen fibers, and interspersed scattered demyelinated axons. Macrophages with lipid droplets may be present for months in chronic lesions (Fig. 7). Interestingly, chronic plaques that may be partially remyelinated, prevalently by Schwann cells, may be side by side with more acute lesions characterized by the presence of numerous macrophages, completely filled with myelin debris (Fig. 8). This picture is again strongly reminiscent of the situation in human MS, where, as recently pointed out by Prineas et al. (1993), new lesions are most often present in or in close proximity to preexisting lesions, a feature of recurrent disease.

DBA mice. These strains (DBA/1 and DBA/2) are clinically very susceptible to TMEV-induced white matter pathology; however, lesions in them are less destructive. Disorganization of white matter architecture is much less severe than in SJL mice, and demyelination is accompanied by only mild axonal alterations. The overall result is the production of extensive areas of denuded axons, which appear otherwise normal, and in close proximity to one another (Figs. 9, 11). In addition, in contrast to SJL/J mice, most macrophages do not remain unabated for long periods of time in the diseased white matter (Fig. 9), and remyelinated axons are often observed in both anterior and lateral columns starting just a few weeks after infection. Remyelination is accomplished by both oligodendrocytes and Schwann cells, but in many areas oligodendrocytes prevail (Figs. 10, 12).

NZW and RIII/SJ mice. Lesions in these susceptible mice are the most exquisitely demyelinating when compared with the above strains. As in other mice, lymphoid infiltrates are first observed in meninges and perivascular parenchimal spaces, followed soon by demyelination in concomitance with the presence of macrophages. The degree of inflammation is, however, less than in DBA and especially SJL/J mice, and, in particular, macrophages appear to be quickly removed from demyelinated lesions. The end result of this different pattern of lesion production is the presence, on light microscopy, of large aggregates of demyelinated axons, often back to back without intervening glial processes (Fig. 13). Also, in these mice remyelination is quite active, and, in contrast to the above strains, it is mainly carried out by oligodendroglial cells (Figs. 13–15).

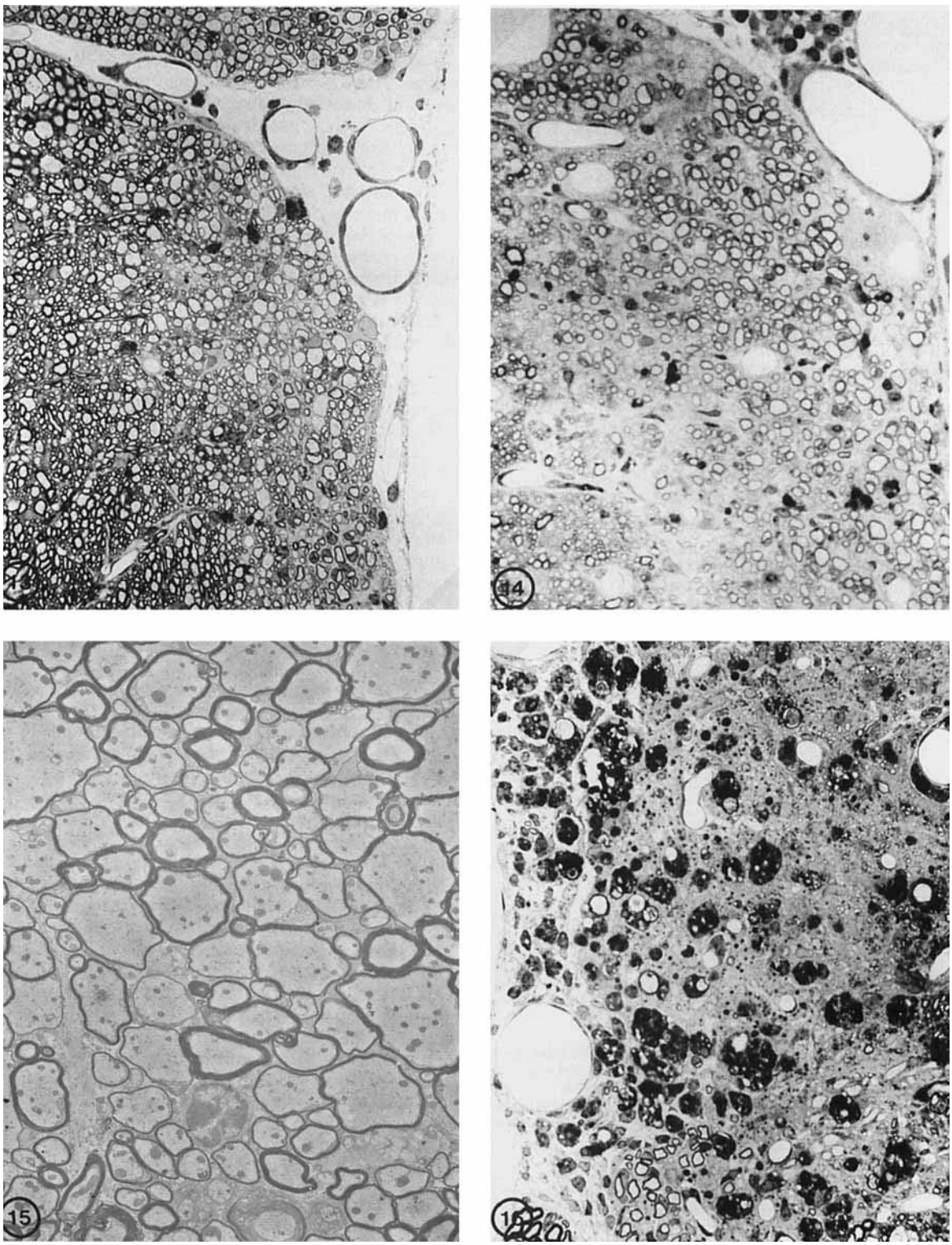
The above findings suggest a gradient of severity of lesions in different strains which, clinically, may be similarly susceptible. The morphological parameter that seems to best correlate with such pathological differences is the number of macrophages and the extent of time that these cells linger in the diseased tissue. There is in fact a clear gradient in terms of macrophage behavior in these different strains, with the SJL/J mice being the strain showing the most numerous and persistent macrophages in their tissues and, accordingly, the most destructive lesions for both myelin and axons. Remyelination is sporadic in these mice. NZW and RIII/SJ mice are at the other end of the spectrum, with fewer macrophages, which exit the scene more quickly, and with the most purely demyelinating lesions than other susceptible strains. In parallel with less severe destruction, remyelination in these animals is very active. One could argue that macrophages in SJL mice are more numerous and persist longer because lesions are larger. This does not seem to be the case, since lesions showing myelin breakdown and demyelination in both NZW and RIII/SJ mice are at least as large as in SJL mice, often coalescing to occupy entire anterior and lateral columns. It does not appear, therefore, that macrophages are held longer in one strain because of more debris to clear than in other strains; rather, it is possible that differences in immune regulatory responses in different strains may account for different macrophage behavior, which in turn may affect the severity of lesions. These issues are under investigation and are not discussed further in this report.

Fig. 9. An acute to subacute lesion in a DBA/2 mouse infected with BeAn virus. This picture illustrates the different pathological outcome in this murine strain, when compared with the SJL mouse. A few macrophages are present in this lesion, but their presence is far less dramatic than in SJL mice (compare with Fig. 2). In contrast with the situation in SJL/J mice, and more similarly to human MS, demyelinated axons in these lesions are much closer together, so that the white matter architecture is respected and there is no significant expansion of the intercellular space. One micron thick, Epon-embedded section, stained with toluidine blue, 6 weeks after infection. ×275.

Fig. 10. A subacute lesion in a DBA mouse 8 weeks after BeAn infection. Numerous demyelinated axons in this lesion are acquiring new myelin recognizable as very thin periaxonal rings. A few axons are in contact with Schwann cells, but the majority are remyelinated by oligodendrocytes. One micron thick, Epon-embedded section, stained with toluidine blue. ×250.

Fig. 11. An ultrastructural view of a demyelinated area in a DBA mouse, comparable to that seen in Fig. 9. Demyelinated axons are in close proximity to each other, and there is very little expansion of the extracellular space. ×24,000.

Fig. 12. Ultrastructural appearance of a demyelinated field in a DBA mouse infected with BeAn virus. Central-type myelin is being formed around these axons, which are arranged in close proximity to each other. Small glial processes are in between some of them. The disorganized myelin figure at the top could be interpreted either as fresh demyelination or, more likely, as an abherrant remyelinating attempt. ×27,000.



Figs. 13–16.

Immunological Studies

The above mentioned pathological features of inflammatory demyelination are, per se, strongly suggestive of an immune-mediated mechanism of myelin injury in this model (Dal Canto and Lipton, 1975). The observation that nonspecific immunosuppression using cyclophosphamide and/or anti-thymocyte serum resulted in a dramatic reduction in the extent of mononuclear cell infiltration and prevention of demyelination was the first firm experimental datum in favor of the immune hypothesis (Lipton and Dal Canto, 1976; Roos et al., 1982). Later studies have supported this notion. For instance, susceptibility fails to correlate with serum anti-TMEV antibody titers (either total or neutralizing antibody) (Clatch et al., 1986, 1987a,b) or with the amount of TMEV plaqued from the CNS during the first 30–40 days postinfection (Clatch et al., 1985, 1987a,b), data against a direct cytolitic effect of the virus on CNS cells.

The mononuclear cell nature of the inflammatory infiltrate and linkage of strain susceptibility to both H-2 and T cell receptor genes, mentioned above, are suggestive of a T cell–mediated pathogenic process. Inhibition of antigen presentation by treatment with monoclonal anti-MHC class II antibodies resulted in inhibition of chronic paralysis and reduced inflammation and demyelination when given concomitant with or after the establishment of persistent infection (Friedmann et al., 1987; Rodriguez et al., 1986b). Direct evidence indicating that the pathologic process is T cell–dependent was provided by the failure of adult thymectomized, irradiated, bone marrow–reconstituted susceptible SJL/J mice to develop clinical or histologic signs of TMEV-induced demyelinating disease following infection with the BeAn strain of virus (Gerety et al., 1994). In addition, transfer of TMEV-specific T cell blasts (either polyclonally activated from lymph node cells of TMEV-primed mice or long-term T cell lines) results in an increase in the incidence and severity of disease in syngeneic recipients infected intracerebrally with a suboptimal dose of TMEV but not in uninfected recipients (Gerety et al., 1994).

The effector nature of the T cell subset(s) responsible for demyelination is a matter of some controversy and may depend on the TMEV substrain used to infect susceptible mice. Rodriguez et al. (1985, 1986a,c, 1993) have published extensively on the possible role of $CD8^+$ cells in TMEV infection when using the DA strain of the virus. We have focused our attention mainly on the $CD4^+$ T lymphocyte, which in the infection produced by the BeAn strain of TMEV appears to have a central role in pathogenesis. In the following discussion, therefore, we shall limit our comments to the $CD4^+$ cell in BeAn infection.

Welsh et al. (1987) found that CBA mice depleted of total T cells or $CD4^+$ cells after infection with the BeAn strain of TMEV but prior to the onset of clinical signs of demyelination exhibited approximately 50% reduced disease incidence. Similarly, studies with SJL/J mice in our laboratory have shown that monoclonal antibody depletion of $CD4^+$ cells, beginning 10–14 days after infection with the BeAn strain of TMEV, resulted in a decreased incidence of demyelinating disease and a less severe clinical course as well as a slower onset of disease in those mice which eventually became clinically affected (Gerety et al., submitted). Flow cytometric analysis of the Percoll-purified mononuclear cells in highly susceptible SJL/J mice revealed that approximately half of the cells infiltrating the CNS were macrophages (i.e., esterase positive) and approximately half were T cells (as assessed by staining with anti-CD3 and anti-TcR antibodies) (Clatch et al., 1990). Fifty to sixty percent of the infiltrating T cells are $CD4^+$ and 40–50% are $CD8^+$. More recently, analysis of the CNS-infiltrating T cells assayed at varying times ranging from 10–120 days post-TMEV infection has revealed that essentially all of the recently activated cells (i.e., those bearing IL-2 receptor) are $CD4^+$ (10–20% of the total cells), while less than 1% of the infiltrating $CD8^+$ cells bear IL-2R, suggesting a major role for $CD4^+$ cells in the demyelinating process (unpublished observation).

Relationship Between TMEV-Induced Demyelination and EAE

The similarity of pathological alterations in TMEV-induced demyelinating disease and EAE, the classical model for autoimmune demyelination, suggested initially that demyelination in TMEV infection could follow a process of secondary anti-myelin autosensitization, thus resulting in a virally induced EAE-like model. There are several reasons why we believe this is not the case, and in order to analyze such reasons we shall now briefly review the murine CR-EAE model and discuss its relationship to the TMEV model.

Fig. 13. The result of a nondestructive type of inflammatory attack is illustrated in this photograph from an RIII/SJ mouse, 12 weeks after BeAn infection. The architecture of the spinal cord is completely preserved, and remyelination has taken place in an orderly fashion, although myelin sheaths are obviously too thin. A picture like this corresponds to the typical shadow plaque, which may be seen in human MS. One micron thick, Epon-embedded section, stained with toluidine blue. ×275.

Fig. 14. A similar nondestructive outcome is observed in this NZW mouse, 10 weeks after BeAn infection. Inflammatory cells are still present in the leptomeninges, but the spinal cord parenchima appears clean of macrophages. The large demyelinated lesion is being remyelinated almost completely by oligodendrocyte activity, another picture reminiscent of human MS shadow plaques. One micron thick, Epon-embedded section, stained with toluidine blue. ×290.

Fig. 15. The ultrastructural appearance of a field similar to the one in Fig. 13. Essentially every axon has been remyelinated by oligodendrocytes, and the architectural relationship between the axons is essentially normal. This picture is in striking contrast to the end result of demyelination in SJL mice similarly treated. ×6,000.

Fig. 16. Spinal cord from an SJL mouse with adoptive passive EAE, produced by cells sensitized to whole myelin. Macrophages are in moderate numbers. Numerous demyelinated axons are seen in the lower part of the picture, some of which are being remyelinated by Schwann cells, despite the fresh inflammatory activity. The background is moderately gliotic. Two weeks after cell transfer. One micron thick, Epon-embedded section, stained with toluidine blue. ×275.

EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE)

EAE is a $CD4^+$ T cell–mediated, myelin antigen-specific autoimmune process leading to the development of inflammatory demyelination of brain and spinal cord in several animal species. It is probably the closest experimental counterpart of human postinfectious or postvaccinial enecephalomyelitis (Adams, 1959). For decades, it has also been considered the best experimental model for human MS, although in most species EAE does not reproduce the typical MS features of chronicity and/or relapsing-remitting disease activity. In 1981, a mouse model of chronic relapsing EAE was introduced (Brown and McFarlin, 1981), and, because of the well-defined immunologic and genetic profiles of the murine system, it has become the most popular autoimmune model for human MS (Brown et al., 1982; Fritz and McFarlin, 1989; Lublin et al., 1981; McRae et al., 1992; Mokhtarian et al., 1984; Pettinelli and McFarlin, 1981; Trotter et al., 1987; Tuohy et al., 1989; van der Veen et al., 1989)

As for TMEV, there are murine strains which are susceptible to EAE, as there are strains which are resistant. It is of interest that susceptible mice may respond to different epitopes of either MBP or PLP, the two most effective autoantigens in inducing the disease (Fritz and McFarlin, 1989; Trotter et al. 1987). We have been studying this model for several years, particularly in the SJL/J mouse (Kennedy et al., 1988, 1990).

Animal Sensitization

Animals may be sensitized to either whole spinal cord homogenate (Brown and McFarlin, 1981), to purified MBP or PLP (Fritz and McFarlin, 1989; Trotter et al. 1987), or to the specific peptides which correspond to the encephalitogenic portion of these different molecules for a particular strain (Fritz and McFarlin, 1989; Tuohy et al., 1989). Alternatively, to focus on the effector phase of the disease, one can transfer sensitized, antigen-specific $CD4^+$ cells into syngeneic animals, thus producing passive EAE (Fallis et al., 1989; Pettinelli and McFarlin, 1981). This method of disease induction is the preferred method since it gives a closely reproducible pattern of disease in terms of timing of clinical presentation, pathological features, remissions, and relapses. We shall focus on this form of EAE for the following discussion.

Clinical Disease

After adoptive transfer of $CD4^+$ neuroantigen-specific T lymphocytes, mice come down with initial clinical signs at 6–8 days after sensitization. The clinical presentation is characterized by a waddling gate, rapidly progressing to ataxia, hind leg weakness, paralysis, and urinary incontinence. This first bout of disease lasts generally 2 weeks, after which time mice recover to a certain degree. The following relapse can be expected after about 4 weeks of injection. More remissions and relapses follow, although less predictably than the first relapse.

Pathological Alterations

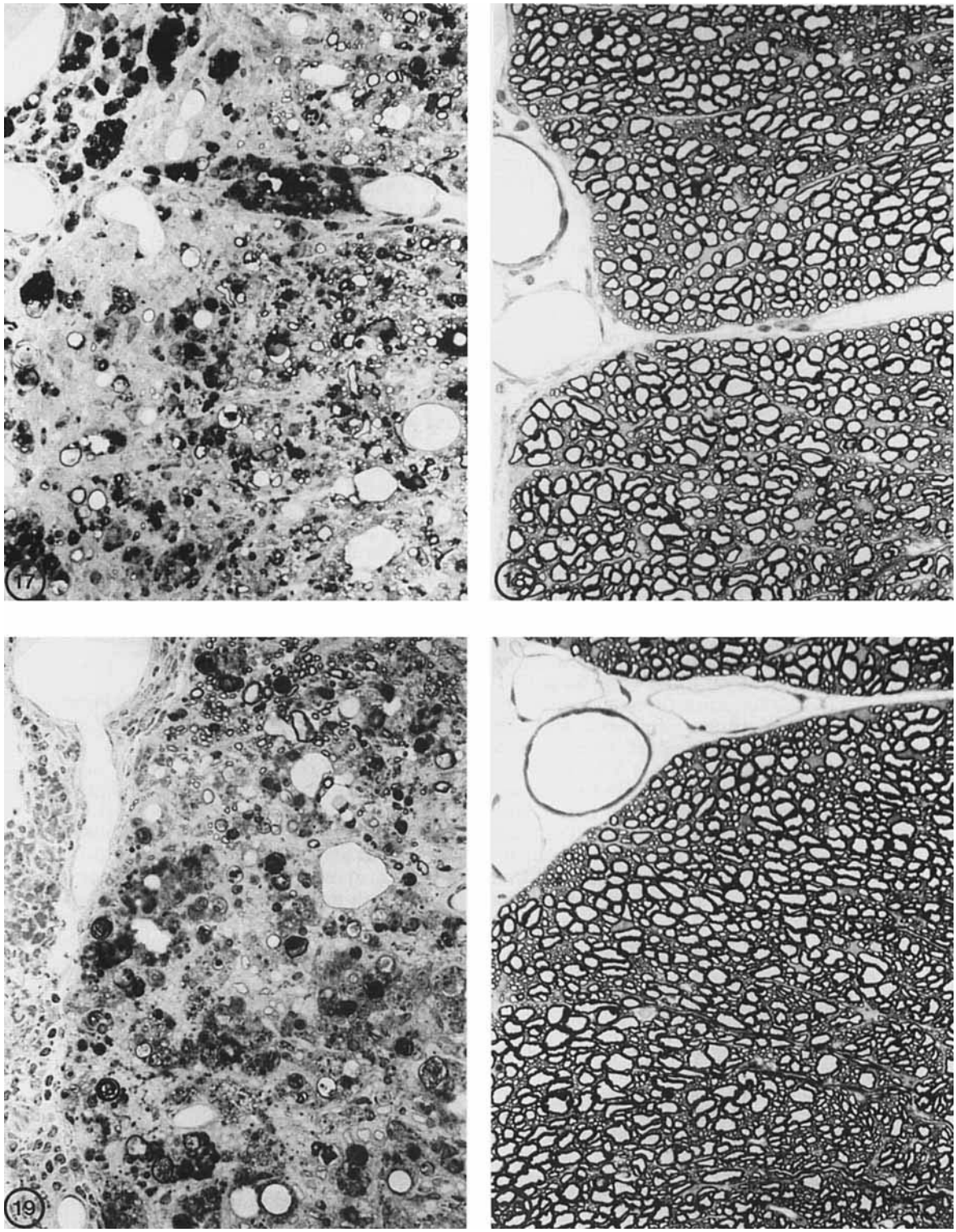
As previously described by Raine et al. (1984), the pathological features of adoptively transferred EAE show less destructive activity than those of active EAE, particularly in regards to axonal pathology (Brown et al., 1982). However, when compared to other EAE models, such as the chronic guinea pig model (Raine et al., 1974), CR-EAE in the mouse is generally more destructive to white matter, with resultant more marked reactive gliosis. From this point of view, therefore, the CR-EAE model is intermediate for its severity of destruction between the chronic guinea pig model of EAE and TMEV infection in the SJL/J mouse. Inflammatory infiltrates precede white matter changes and consist of lymphoid infiltrates in meninges, perivascular spaces, and eventually the parenchyma itself. Macrophages are numerous (Fig. 16), although rarely as numerous as in TMEV infection in SJL/J mice, and, at least in our material, lesions become generally confluent to involve large areas of both anterior and lateral columns. Correspondence with clinical evidence of remitting-relapsing activity is generally good, but pathological changes often precede the clinical presentation of signs by a few days. During the remitting phase of the disease, inflammation subsides and glial processes take much of the space left behind them. Naked axons are present in moderate numbers, and, in our material, they are generally separated by thick collars of glial processes, since, as previously noted, mild to moderate axonal destruction has generally taken place. As in Theiler's infection, macrophages are rather reluctant to leave and remain in the tissues for many weeks, even when lymphoid infiltrates have largely subsided (Fig. 17). This is a feature which is also common to human MS, as pointed out by Raine (1991). It is of interest that Theiler's infection in the SJL mouse al-

Fig. 17. Spinal cord from an SJL mouse 5 weeks after induction of passive EAE and sham tolerized. The EAE process is not affected by sham tolerization. At this time point, lesions show decreased inflammatory activity, and almost all cells around vessels in the meninges and in the white matter are macrophages, laden with myelin debris. The moderately gliotic background is consistent with a moderate degree of axonal destruction. One micron thick, Epon-embedded section, stained with toluidine blue. ×275.

Fig. 18. Littermate of mouse in Fig. 17, similarly injected for induction of passive EAE, but tolerized with spleen cells, ECDI-coupled with whole myelin. Tolerance induction has prevented the development of EAE and the spinal cord appears essentially normal. One micron thick, Epon-embedded section, stained with toluidine blue. ×275.

Fig. 19. SJL/J mouse, infected with TMEV and sham tolerized, sacrificed 82 days after infection. The spinal cord shows similar changes to those described in Fig. 2, since sham tolarization has no effect on the disease process. Note how inflammatory activity is still very active at this time point, and the number of macrophages in the tissue. One micron thick, Epon-embedded section, stained with toluidine blue. ×275.

Fig. 20. SJL/J littermate of the mouse in Fig. 19, infected with TMEV but tolerized with TMEV-coupled spleen cells by ECDI. At the same time points as Fig. 19, no disease is present. One micron thick, Epon-embedded section, stained with toluidine blue. ×275.



Figs. 17–20.

ways produces a progressive course, since new lesions are constantly being produced, often extending old lesions, even after many months of infection. Chronic relapsing EAE, on the other hand, despite the rather destructive nature of its lesions, has periods in which inflammatory activity is greatly decreased. Clinical remissions, therefore, are recognizable, and when renewed inflammatory-demyelinating activity takes place, this generally heralds a clinical relapse. Both types of presentations are also recognized in human MS.

Remyelinating activity has been observed in CR-EAE lesions by Raine et al. (1984). In our material there was less remyelination than shown in their paper, probably related to the somewhat more destructive nature of lesions in our animals. It is interesting that, when remyelination was observed, it was carried out preferentially by Schwann cells rather than oligodendrocytes. This may again reflect the more severe aspects of inflammation and destruction in our material and recalls quite closely the situation in TMEV-infected SJL/J mice, where oligodendrocyte remyelination is very sparse.

Studies of Pathogenetic Differences Between the Two Models

As previously mentioned, the similarities between EAE and Theiler's virus infection in SJL/J mice suggested the possibility that TMEV-induced demyelination was perhaps also dependent on autosensitization to myelin antigens. We first looked at this possibility by comparing the effects of sera and cells from EAE animals and TMEV-infected mice in myelinating and myelinated organotypic cultures (Barbano and Dal Canto, 1984). Cells were first expanded by culture in the presence of myelin antigens. While EAE sera and cells inhibited myelination and were able to demyelinate organotypic cultures, no effect was noted with sera and cells from TMEV-infected animals. The same sera and cells, in addition, were not able to transfer disease in vivo. These studies strongly suggested that demyelination in Theiler's infection was not due to an EAE-like mechanism.

More recently, the powerful technique of tolerance induction to a specific antigen has made it possible to assess the role that an antigen may play in an immune-mediated disease process. We have been conducting tolerization studies in both EAE and TMEV to compare these two models, particularly in relation to the identity of the antigen(s) responsible for immune activation. As we shall now briefly summarize, such studies also support the notion that demyelination in TMEV infection is not due to secondary development of an anti-myelin autoimmune response.

Tolerization studies of EAE. Tolerance induction is accomplished by the intravenous injection of spleen cells coupled to the appropriate antigen by the coupling agent ethyl-carbodiamide (ECDI) (Kennedy et al., 1988, 1990). It appears that ECDI treatment of immune cells interferes with important secondary signals from the antigen-presenting cell to the effector cell, so that the effector cell, rather than being stimulated by contact with antigen, is made anergic. It has been suggested that tolerance induction results in a downregulation of IL-2 gene expression in effector Th1 cells (Jenkins et al., 1987). These constitute a subpopulation of $CD4^+$ T cells involved in the development of DTH responses. The state of clonal anergy resulting from the tolerization regimen is long-lasting, antigen-specific, MHC Class II–specific, and dose-dependent (Kennedy et al. 1988, 1990; Miller et al. 1992; Tan et al., 1992).

Tolerance induction to neuroantigens in SJL/J animals results in abrogation of EAE (Figs. 17, 18). Such downregulatory activity is equally effective in both disease induction and in disease expression. Active EAE, produced by sensitization with whole spinal cord, can be prevented by i.v. injection with spinal cord–coupled spleen cells or with PLP-coupled spleen cells but not with MBP-coupled cells, suggesting that in whole spinal cord homogenates the dominant encephalitogenic epitope is PLP rather than MBP. On the other hand, MBP-induced EAE can only be prevented by tolerization with MBP-coupled spleen cells. Similarly, in passive EAE, tolerization is able to prevent disease only when spleen cells are coupled with either whole myelin or the same antigen against which the cells used to transfer the disease had been induced. Importantly, EAE can be prevented by injecting spleen cells coupled to peptides from myelin antigens, such as MBP 84–104 or PLP 139–151, which are major encephalitogenic peptides in the SJL/J mouse, when adoptive EAE is induced by the respective antigens (Miller et al., 1992). Of great interest have also been studies of tolerization in animals with established EAE. They have shown that tolerance induction does not only prevent the induction of EAE, but, once EAE has been established, subsequent relapses may be prevented (Tan et al., 1991). Interestingly, such tolerization studies in established chronic relapsing EAE have also shown that subsequent relapses of EAE in SJL/J mice may be due to different antigens than the one originally used for disease induction. In other words, these studies have shown that an antigen shift may occur during a demyelinating disease with autoimmune pathogenesis, a finding that may have relevance to human MS as well. Of paramount importance is the fact that tolerance induction prevents the clinical and pathological expressions of disease by abrogating the development of a DTH response to neural antigens. This clearly establishes the role of the DTH response to myelin antigens in the induction of disease. The Th1 subset of $CD4^+$ lymphocytes has been recognized as the primary cells capable of mediating such responses.

Tolerization Studies in TMEV Infection

Our previous studies have shown that susceptibility strongly correlates with the development of chronic, high levels of TMEV-specific DTH responses (Clatch et al., 1985, 1986, 1987a,b). DTH in BeAn-infected susceptible SJL/J mice develops within 10–14 days postinfection, preceding the onset of clinical signs, and remains at high levels for at least 6 months postinfection. TMEV-specific DTH in SJL mice is mediated by $CD4^+CD8^-$ T cells and is restricted by $I\text{-}A^s$ determinants. Subsequent studies have strongly supported an immunopathologic role for the Th1 subset of $CD4^+$ T

cells in the pathogenesis of TMEV-induced demyelinating disease. Firstly, TMEV-susceptible mouse strains make an anti-TMEV antibody response which is dominated by the IgG2a isotype, whereas anti-viral IgG1 predominates in resistant strains (Peterson et al., 1992, 1993). The IgG2a and IgG1 isotypes are regulated by Th1- and Th2-derived lymphokines, respectively (Finkelman et al., 1990; Mosmann and Coffman, 1989; Snapper and Paul, 1987). Lastly, we recently demonstrated that the systemic transfer of an SJL/J-derived, TMEV-specific $CD4^+$ T cell line resulted in exacerbation of the incidence and severity of disease in mice infected intracerebrally with suboptimal amounts of TMEV, demonstrating the immunopathologic potential of $CD4^+$ cells. This immunopathologic, $I\text{-}A^s$–restricted T cell line (sTV1) was shown to mediate DTH and to produce IL-2 and proinflammatory cytokines such as IFN-gamma and lymphotoxin in response to antigenic stimulation indicating that it is of the Th1 subset (Gerety et al., 1994).

Because of the relationship found between strain susceptibility to the disease and the ability to mount a DTH response to TMEV, tolerization experiments were also conducted in TMEV infection. These studies had two main purposes. The first was to find further support to the hypothesis that demyelination in TMEV infection is not an EAE-like occurrence. The second was to strengthen the hypothesis that anti-viral DTH responses may play a major role in the pathogenesis of demyelination.

Tolerance was induced to either myelin antigens or TMEV. As previously noted, autoimmune T cell reactivity against the major myelin proteins, myelin basic protein (MBP) and proteolipid protein (PLP), did not appear to be involved in the induction of demyelination following TMEV infection. Demyelination could not be initiated by transfer of either serum or lymphoid cells from TMEV-infected donors to normal, non-TMEV-infected recipients (Barbano and Dal Canto, 1984). Neither MBP- or PLP-specific T cell responses were detected in SJL/J mice at any time following intracerebral inoculation with TMEV (Miller et al., 1987). T cells from TMEV-infected mice also do not respond to the peptide fragments of MBP (amino acids 91–104) and PLP (amino acids 139–151) known to define the major encephalitogenic epitopes in SJL/J mice (Miller et al., 1990). In keeping with those earlier studies, induction of neuroantigen tolerance in SJL/J mice by using whole spinal cord homogenates (a heterogeneous mixture of myelin and nonmyelin CNS antigens) failed to affect the development of clinical and histologic signs of TMEV-induced demyelinating disease or the accompanying virus-specific CMI and antibody responses (Miller et al., 1990). In contrast, as noted earlier, this tolerogenic regimen is extremely effective in reducing the incidence of clinical and histologic signs of MSCH-induced relapsing experimental autoimmune encephalomyelitis and the accompanying neuroantigen-specific DTH responses (Kennedy et al., 1988). On the other hand, induction of tolerance using intact TMEV virions coupled to syngeneic splenocytes, which specifically anergizes virus-specific Th1 responses (Peterson et al., 1993), results in a dramatic reduction in the incidence and severity of demyelinating lesions and clinical disease in SJL/J mice subsequently infected with TMEV (Karpus et al., in press) (Figs. 19, 20).

Collectively, the results of the immunologic analysis of T cell responses in TMEV-infected mice strongly indicate that chronic demyelination can occur in the apparent absence of neuroantigen-specific autoimmune responses and are consistent with the hypothesis that virus-specific DTH plays a major effector role in CNS demyelination following TMEV infection (Clatch et al., 1986). According to this hypothesis, intracerebral inoculation with TMEV leads to an initial viremia followed by a persistent, low-level CNS infection which can last for virtually the lifetime of the animal (Lipton et al., 1984). As a consequence of this infectious process, MHC class II–restricted, TMEV-specific Th1 cells clonally expand, either in the periphery and/or locally within the CNS. Subsequent release of proinflammatory cytokines (IFN-gamma and LT/TNF-beta) by the Th1 cells in the CNS would then lead to the recruitment and activation of monocytes and macrophages which cause myelin destruction by a terminal nonspecific bystander mechanism (Dal Canto and Lipton, 1975). This hypothesis is also consistent with the characteristic mononuclear cell infiltrates associated with destructive demyelinating lesions and would account for the chronic attraction of host monocytes/macrophages, the cells in which TMEV is known to primarily persist (Clatch et al., 1990), to the CNS.

The final pathway leading to myelin destruction, therefore, may be very similar in both EAE and TMEV-induced demyelination—that is, a process of delayed type hypersensitivity. The major difference, however, is that in EAE the driving antigen(s) is clearly derived from myelin itself, while in TMEV infection, at least in the first several weeks, the driving antigens are from the infecting virus. In human MS, it is not clearly established which antigen(s) is responsible for exciting the immune response eventually leading to myelin destruction. It is hypothesized that a viral infection may indeed initiate the disease process (Poser, 1993), perhaps in a manner similar to TMEV infection. Whether later on autoimmune responses misdirected against myelin develop is still debated, and there is considerable controversy about a proposed restriction of T cell receptor usage by MBP-specific T cells derived from MS patients (Martin et al., 1992; Chou et al., 1992). On the other hand, it is still possible that a virus(es) may play the most important role in inducing myelin pathology, in a manner similar to TMEV-induced demyelinating disease.

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