Mechanisms of Virus-Induced Demyelination and Remyelination[a]

MOSES RODRIGUEZ

Department of Neurology and Immunology
Mayo Clinic and Research Foundation
Rochester, Minnesota

Animal models of virus-induced demyelination and remyelination have provided one important piece of evidence to suggest that multiple sclerosis is the result of immunopathology induced by a virus.[1] These models provide the framework to study the potential interaction between the immune system, persistent viruses, and glial cells. This review will address major mechanisms considered to be important in the pathogenesis of virus-induced demyelination including:

1. Direct viral cytopathologic effects on oligodendrocytes
2. Virus-induced autoimmune demyelination
3. "Bystander" demyelination
4. Immune-mediated alteration of viral tropism for oligodendrocytes
5. Immune-mediated destruction of persistently infected oligodendrocytes.

Each mechanism will be illustrated by various examples of virus infection. Special emphasis will be given to Theiler's murine encephalomyelitis virus(TMEV)-induced demyelination, a model that exemplifies many potential mechanisms of myelin destruction. In addition, the factors that control new myelin formation after virus-induced demyelination will be discussed.

DEMYELINATION

Direct Cytopathology of Oligodendrocytes by Virus

One of the best examples of a virus causing demyelination by direct lytic infection of the myelin-producing cell is the JHM virus, a neurotropic strain of mouse hepatitis virus.[2-6] This coronavirus produces demyelination in susceptible BALB/c mice within the first week of infection. Demyelination in this model is not temporally related to the presence of perivascular inflammatory cells,[2,3] and immunosuppression with cyclophosphamide fails to diminish demyelination in the mouse, strongly suggesting that

[a]This work was supported in part by grants from the National Multiple Sclerosis Society (RG 1878-A1) and Searle Foundation. Moses Rodriguez is the recipient of a Teacher Investigator Award (NS-00849) from the National Institutes of Health.

immune mechanisms are not involved. The virus can infect neurons and astrocytes,[6] but oligodendrocytes appear to be the principal target.[2] Experiments by Powell and Lampert[4] demonstrated oligodendrocytes containing intracisternal virions. Virus buds from cytoplasmic vacuoles, leading to pathologic alterations of oligodendrocytes, which then result in abnormal glial connections with myelin sheaths and syncytia formation.

A characteristic feature of JHM-induced demyelination is the rapid recovery of infected animals,[2,5] resulting from proliferation of surviving oligodendrocytes and remyelination of previously demyelinated axons.[5] Myelin sheaths are almost completely restored within 2 to 3 months of infection. Prominent remyelination, observed with this model, indicates that oligodendrocytes within the central nervous system (CNS) have an intrinsic capacity for myelin repair, even when primary injury is directed at the myelin-producing cell. As will be discussed, the extent of remyelination in this model may indicate that immune mechanisms are not important in JHM-induced demyelination.

Papovaviruses also appear to cause demyelination by direct injury of oligodendrocytes.[7-10] The JC virus, a member of the papovavirus family, causes progressive multifocal leukoencephalopathy in immunosuppressed patients.[8] This rare demyelinating disease was seen in patients with lymphoma and leukemia,[7] but it has emerged as an important complication of human immunodeficiency virus (HIV) infection.[9] Pathologically, it is characterized by multiple patches of noninflammatory demyelination without relation to blood vessels.[7] Infected oligodendrocytes are easily recognized by their enlarged nuclei containing papovaviruses. Astrocytes are transformed and develop bizarre, hyperchromatic nuclei. Recent experiments using *in situ* hybridization clearly demonstrated the remarkable propensity of this virus for oligodendrocytes.[10]

Virus-Induced Autoimmune Demyelination

An attractive hypothesis in virus-induced demyelination is that virus infection can trigger a destructive host immune response to self antigens.[11] A basis for this idea comes from observations in patients with postinfectious encephalomyelitis in which perivenular demyelination develops 2 to 3 weeks after virus infection including measles or vaccinia infection and, to a lesser extent, varicella or rubella infection. The pathologic features closely resemble those of acute experimental autoimmune encephalomyelitis.[11] This finding raises the possibility that viruses can cause primary damage to oligodendrocytes or myelin sheaths, or both. This then results in the release of "self" myelin antigens that would be recognized as foreign by immunocytes. This hypothesis would be supported by the demonstration of cellular or humoral immune responses, or both, to myelin antigens after virus infection and by passive transfer of pathologic abnormalities into naive recipients by immune serum or lymphocytes.

Probably the best example of virus-induced autoimmune demyelination is coronavirus infection in rats.[12-13] Watanabe *et al.*[13] inoculated rats with a murine coronavirus and observed late demyelinating disease characterized by perivascular lymphoid infiltration. Early in the disease, viral antigen was found primarily in glial cells in association with small demyelinating plaques. As the animals recovered from the initial infection, late demyelinating disease developed and was associated with intense inflammatory infiltrates. Lymphocytes from infected rats were sensitized against myelin basic protein (MBP) and virus antigen.[13] Lymphocytes from Lewis rats recovering

from infection were cultured *in vitro* in the presence of myelin basic protein and injected intravenously in naive syngeneic rats. In a few days, mild clinical disease and perivascular inflammatory infiltrates resembling EAE developed. Interestingly, demyelination was not detectable.

Thus far, autoimmune demyelination has not been confirmed in any other viral model. This hypothesis was tested in the demyelinating disease induced by Theiler's virus (TMEV), a picornavirus that results in chronic immune-mediated demyelination.[14,15] Barbano and Dal Canto[16] failed to produce demyelination *in vitro* when isogenic organotypic brain cultures were exposed to serum or splenocytes from mice persistently infected with TMEV. In addition, disease could not be transferred into naive recipients when splenocytes from infected mice were incubated with myelin basic protein. The studies agree with those of Lampert *et al.*[17] who concluded that MBP-sensitized cells are not elicited in TMEV infection. Also, Miller *et al.*[18] showed that class II-restricted autoimmune responses against syngeneic spinal cord homogenate or MBP are not demonstrable in susceptible SJL/J mice. Finally, experiments using sensitive immunoblotting techniques failed to demonstrate within cerebrospinal fluid an immune response to myelin antigens.[18a] Thus, data do not support a critical role for autoimmune demyelination in TMEV disease.

One other model in which autoimmune demyelination remains a possibility, however, is the late phase of canine distemper virus (paramyxovirus) encephalitis.[19,20] This natural disease in dogs is characterized by central nervous system symptoms and signs in the acute viral phase. Similar to measles virus, the acute syndrome can cause lymphopenia to develop in dogs. Intracellular virus in the absence of inflammatory cells has been demonstrated in acute demyelinating lesions. In contrast, the late demyelinating disease is associated with perivascular cuffs of inflammatory cells, and antimyelin antibodies develop before the onset of symptoms.[20] Thus far, demyelination has not been obtained in rodents infected with the canine distemper virus, making it very difficult to formally test the autoimmune hypothesis in this model.

Bystander Demyelination

Considerable thought has been given to the concept that myelin may be injured "nonspecifically" as a result of an immune response within the nervous system,[21,22] and it would help explain why different viruses may result in myelin destruction.[1] This hypothesis suggests that T cells, macrophages, or both, in reacting to a viral antigen, secrete factors that cause demyelination. For example, myelin is vulnerable to neutral proteases, including plasminogen activator, which can be secreted by activated macrophages.[22] Some experiments suggest that demyelination can occur after local injection of purified protein derivative in the spinal cord of animals previously sensitized to this antigen.[21] However, other similar experiments failed to show a "bystander effect" in the peripheral nervous system.[23] It is possible that "bystander" demyelination may be important in augmenting myelin destruction, especially in a host with latent hypersensitivity to myelin.

The bystander hypothesis was considered by Clatch *et al.*[24] to explain demyelination induced by Theiler's virus. They propose that as a consequence of persistent virus infection, TMEV-specific precursor delayed hypersensitivity (DTH) cells are triggered to expand within the brain. These cells release lymphokines which would lead to recruitment of activated macrophages. Factors released by DTH cells or macrophages

would then nonspecifically destroy myelin. In support of this hypothesis is the close relation between skin DTH response and susceptibility to TMEV infection. Also, virus persists within macrophages in the CNS which may predispose to bystander demyelination.[25] Whether this factor contributes significantly to TMEV-induced disease is not yet clear, but there are data to suggest that this may not be the primary mechanism. (1) TMEV-induced demyelinating disease is controlled in part by genes within the major histocompatibility complex.[26-29] However, the disease maps within the H-2D region[27,29] which controls class I-restricted immune responses. If DTH-mediated bystander demyelination were important, restriction to class II genes could be hypothesized. (2) Treatment of TMEV-infected mice with aminomethylcyclohexane carboxylic acid (AMCHA), ϵ-amino caproic acid (EACA), and *p*-nitrophenyl guanidlinobenzoate (NPGB), which are inhibitors of plasminogen activators and other neutral proteases, fail to suppress TMEV demyelination, even though they diminish demyelination in EAE.[30] Also, pepstatin, an acid protease inhibitor that interferes with cathepsin D, fails to diminish TMEV demyelination.[30] (3) Cyclosporin A fails to diminish demyelination once the disease process is established,[30] indicating that effectors dependent on the production of interleukin-2 are not involved in myelin destruction during late disease. (4) Susceptibility to demyelination does not correlate with proliferative responses of class II-restricted viral antigens.[24] (5) Demyelination occurs in nude mice that are deficient in DTH type responses.[31,32] (6) Demyelinating disease can be suppressed by treatment with mAb to Lyt2 (directed at class I-restricted T cells), whereas mAb to L3T4 (directed at class II-restricted T cells) increases demyelination.[33] (7) Finally, bystander demyelination runs counter to most human neuropathologic observations, because demyelination is not present in most inflammatory responses to CNS viruses.[34] This finding suggests that the presence of primary demyelination in the context of inflammation implies a more specific cellular or humoral reaction directed against virus, myelin, or oligodendrocytes.

Immune-Mediated Alteration of Viral Tropism for Glial Cells

Nitayaphan *et al.*[35,36] proposed a unique hypothesis in which immune cells could change the surface structure of a virus so that it has more propensity to infect myelin-producing cells. Using Theiler's virus to test this concept, they found that proteases secreted by macrophages can cleave one of the major structural proteins of TMEV (VP1) and thereby disrupt an epitope important in neutralization.[35,36] This could promote viral persistence and subsequent infection of oligodendrocytes. Serum from mice with early disease is less effective in neutralizing VP1-cleaved virus than VP1-uncleaved virus. Therefore, immune cells could be critical in demyelination by producing factors that change the structural properties of viruses rather than in mediating disease.

This hypothesis may apply to visna infection in which virus is able to escape host defense mechanisms. This retrovirus causes a slow natural disease of sheep involving the lungs and the CNS.[37] Pathologically, there is subacute encephalitis in which virus antigen is found primarily in macrophages and demyelination is associated with inflammatory infiltrates.[38] A unique aspect of the disease is the failure to neutralize virus by serum as a result of viral "antigenic drift."[39] Virus isolates from sheep years after infection are antigenically different from input virus. It is possible that factors secreted by macrophages may alter virus and contribute to subsequent "antigenic drift."

Immune-Mediated Destruction of Persistently Infected Oligodendrocytes

Humoral or cellular immune mechanisms may play a role in injuring oligodendrocytes that have been infected by virus.[40] This hypothesis implies the expression of viral antigens or viral-induced "novel" antigens on the surface of myelin-producing cells. Humoral mechanisms may interact with virus antigens on oligodendrocytes, resulting in immunoglobulin-directed killing, injury by complement, antibody-dependent cell-mediated cytotoxicity, or activation of macrophages through binding of Fc receptors. Cellular immune mechanisms would depend on the recognition of processed viral polypeptides or intact structural viral protein by class II- or class I-restricted T cells in the context of major histocompatibility complex (MHC) glycoproteins.

An example of humoral-mediated destruction of infected oligodendrocytes is subacute sclerosing panencephalitis.[41] This persistent measles virus infection is characterized by infection of neurons and oligodendrocytes. A constant feature of the disorder is high titers of anti-measles antibody in the spinal fluid and brain of infected patients. Lysis of infected oligodendrocytes is associated with antibodies that bind to the nucleocapsid of the virus.[41] Studies in tissue culture have suggested a mechanism by which virus persists in the presence of a competent immune response. If virus antigens are expressed on the cell surface, then lysis of infected cells occurs in the presence of antibody and complement. However, if measles-infected cells are cultured in the presence of antibody without complement, antigens are "modulated" off the cell surface, rendering the cell resistant to subsequent immunopathology. Once the antibody is removed, the persistently infected cell will begin to express viral antigens on the surface so that it is once again susceptible to injury by complement and antibody. Thus, the relative concentration of antibody to measles virus or complement, or both, determines if virus will persist in the nervous system or if oligodendrocytes will be killed.[41]

Theiler's murine encephalomyelitis virus infection may prove to be the result of immune-mediated injury of persistently infected oligodendrocytes.[40] Pathologically, the CNS is characterized by perivascular demyelination in association with mononuclear cellular infiltrates.[42] During the first 2 weeks of infection the infiltrates consist primarily of macrophages and class II-restricted T cells (helper and delayed hypersensitivity cells), but as the demyelinating disease progresses (after 21 days of infection), class I-restricted T cells (cytotoxic and suppressor cells) gradually become more numerous.[43] In every example, demyelination is preceded by perivascular inflammation. In addition, immunosuppression by cyclophosphamide,[44,45] antilymphocyte serum,[45] and monoclonal antibodies to Ia[46,47] diminishes the extent of demyelination. Also, Ia antigens are expressed on astrocytes, oligodendrocytes, and endothelial cells after persistent virus infection,[48] suggesting that the demyelinating process is the result of immune mechanisms.

There is, however, strong evidence that oligodendrocytes are infected persistently by virus. Ultrastructural immunoperoxidase experiments have demonstrated virus antigens within oligodendrocytes.[49,50] Paracrystalline arrays of virus were demonstrated within oligodendrocytes of neonates infected by the WW strain of TMEV.[51] The virus readily infects oligodendrocytes in tissue culture,[52,53] and virus antigens can be detected on the surface of these cells.[54] Finally, *in situ* hybridization studies showed a direct correlation between the presence of viral RNA in the white matter and demyelinating lesions.[55–57] Simultaneous immunoperoxidase and *in situ* hybridization assays have shown that 25–40% of cells expressing viral RNA are also expressing antigenic markers specific for oligodendrocytes.[57] Approximately 10% of infected cells are microglia and

macrophages and 5-10% are astrocytes. The identity of the remainder of the cells has not been determined.

There are also strong immunogenetic data that one of the genes that determines susceptibility and resistance to TMEV demyelinating disease maps within the MHC.[26-29] TMEV infection of nonrecombinant H-2 congeneic strains on a common background showed that mice with *s, f, p, r, v,* or *q* haplotypes on C57BL/10 background develop demyelination, whereas mice with *b, k,* or *d* haplotypes are resistant.[28] Infection of mouse strains with congeneic recombinant haplotypes demonstrated that the D region of the H-2 complex determines susceptibility.[29] In addition, the susceptible and resistant gene was mapped to the 3′ end of D^d by using mice with mutations within the D region genes.[29] Because the D region controls class I-restricted immune responses, it suggests an important role of T cells in clearing virus (resistance) or in contributing to demyelination (susceptibility).

Rodriguez *et al.*[40] proposed a hypothesis of immune-mediated demyelination that incorporates the beneficial response to immunosuppression, virus persistence in oligodendrocytes, and the immunogenetic data. This hypothesis suggests that resistance to disease is an active immunologic process. In genetically resistant mice, viral replication may be limited by class I-restricted T cells in the context of H-2D gene products, by natural killer cells (preliminary observations by P. Leibson), or by neutralizing antibody to virus. In genetically susceptible mice, virus antigens may fail to be recognized by T cells in association with class I MHC antigens so that virus is not cleared from the CNS and persists in oligodendrocytes. Antibody to virus may fail to neutralize infection, either because it occurs too late or because antigens are sequestered in the cytoplasm.

Once oligodendrocytes become infected, viral infection may directly induce demyelination, which would explain the presence of demyelination in nude mice without a T-cell response.[31,32] However, in immunocompetent mice, antigens not normally expressed by oligodendrocytes may appear on the cell surface and provide the target for an immune response. The nature of the antigen on the cell surface remains to be determined. It may represent a polypeptide on the surface that resides primarily in an unprocessed form within the cytoplasm. Alternatively, the surface antigen may be a "novel" host-derived protein induced by viral infection. Injury to the oligodendrocytes may occur by humoral mechanisms directed at this antigen or by class I- or class II-restricted T cells recognizing the antigen in the context of MHC glycoproteins. Recent experiments showing suppression of demyelination with monoclonal antibodies to Lyt-2[33] suggest that class I-restricted cells may be one important effector in the demyelinating phase of disease.

REMYELINATION

The factors that control the extent of remyelination after viral-induced demyelination are being evaluated.[58] Some viral infections are characterized by extensive and almost complete myelin repair[5] (i.e., JHM virus infection), whereas in others the extent of remyelination is variable and incomplete[42,59] (i.e., TMEV). Several factors have been considered to explain the extent of new myelin formation after demyelinating conditions, as follows:

1. Degree of oligodendroglial injury or infection
2. Propensity for oligodendroglial proliferation

3. Extent of astroglial "scarring"
4. Intensity of inflammatory response
5. Alteration of demyelinated axon surface
6. Host genetic factors

Of greatest importance is to determine if the original demyelinating process is the result of immune mechanisms. Those disorders in which immune mechanisms play a primary role (chronic experimental autoimmune encephalitis or Theiler's virus) are characterized by abortive attempts at remyelination. In contrast, disorders with minimal immunopathology (JHM infection[2-6] and cuprizone toxicity[60]) show almost completely remyelinated lesions, suggesting that immune factors may be critical in determining the degree of remyelination.

Experiments by Dal Canto and Lipton[42] using the DA strain of TMEV in SJL mice demonstrated abortive attempts at CNS remyelination as early as 21 days after infection. Remyelination was somewhat more prominent in the late phase of chronic infection and was associated with a marked astroglial response. In contrast, experiments with a more attenuated WW strain of TMEV with outbred Swiss male mice resulted in greater remyelination by Schwann cells or oligodendrocytes.[61] This result correlated best with diminution of the inflammatory response in animals infected with WW virus compared to DA virus.

Lang *et al.*[59] undertook a series of experiments in an attempt to promote remyelination after Theiler's virus-induced demyelination. With the observations of Raine and Traugott[62] in mind, a series of experiments were performed to test the hypothesis that immune factors contribute to the extent of remyelination following infection with the DA strain of TMEV. Lang *et al.*[59] found that DAV-infected mice treated by injections of MBP plus galactocerebroside in incomplete Freund's adjuvant (IFA) had areas of extensive remyelination. Similar results were obtained with infected mice injected with spinal cord homogenate (SCH) plus IFA. These results were similar to those of Raine and Traugott[62] in promoting remyelination in guinea pigs with chronic EAE.

Rodriguez *et al.*[64] tested the hypothesis that new myelin formation observed in infected mice treated with myelin components is the result of a humoral factor. Normal syngeneic SJL/J mice were divided into three groups and injected subcutaneously in the flank with a 1-mg dose of SCH in IFA, phosphate-buffered saline (PBS) in IFA (1:1), or PBS alone. Serum was collected and passively transferred into mice chronically infected with DA virus. Of interest is that TMEV-infected animals treated with serum from mice given SCH had extensive areas of remyelination that were 6 to 11 times greater than those in the control groups (FIG. 1). Oligodendrocytes were clustered in groups, suggesting proliferation. The addition of SCH sera to oligodendrocytes grown in tissue culture resulted in three- to fivefold proliferation as measured by the incorporation of tritiated thymidine. This finding suggests that a factor is present in the sera of mice immunized to SCH that promotes new myelin formation and proliferation of oligodendrocytes. The identity of the factor remains to be determined. Preliminary studies suggest that the active factor is in the immunoglobulin fraction of sera. In addition, lymphokines may be important in triggering oligodendrocytes to divide and myelinate.

SUMMARY

Viral models of demyelination and remyelination provide important clues to the pathogenesis of multiple sclerosis. Determining the precise viral polypeptides recognized by T cells during the demyelinating process will be important in understanding

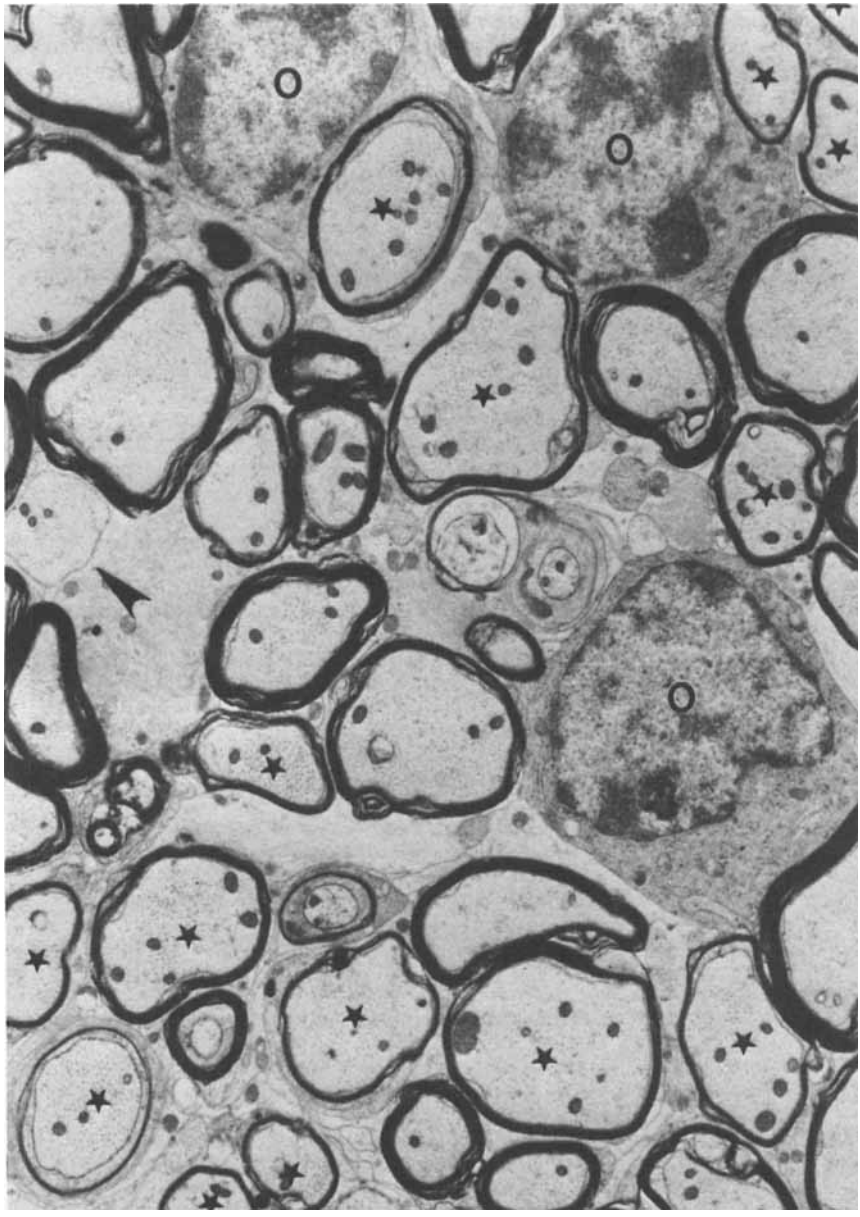

FIGURE 1. Extensive remyelination by oligodendrocytes in the spinal cord of an SJL/J mouse infected with the DA strain of TMEV (6 months) and treated for 1 month with sera from a mouse hyperimmunized to spinal cord homogenate (SCH sera). Note three oligodendrocytes (O) making contact with newly synthesized myelin in the area of remyelination. New myelin formation in the CNS is characterized by abnormally thin myelin sheaths compared to axon diameter (**star**). One demyelinated axon that has not undergone remyelination is shown by the **arrow.** The area of remyelination in mice treated with SCH sera was significantly greater ($p < 0.01$) than that in mice treated with control sera.[64] Similar areas of remyelination were seen in TMEV-infected mice treated with a purified IgG preparation of SCH sera. (Reduced by 35%)

the mechanisms of viral-induced myelin destruction. Isolation, purification, and characterization of factors that promote remyelination and proliferation of oligodendrocytes may provide hope in the treatment of patients with chronic demyelinating disorders.

ACKNOWLEDGMENT

I wish to thank Kathryn A. Jensen for helping to prepare this manuscript.

REFERENCES

1. DAL CANTO, M. C. & S. G. RABINOWITZ. 1982. Experimental models of virus-induced demyelination of the central nervous system. Ann. Neurol. **11:** 109-127.
2. LAMPERT, P. W., K. J. SIMS & A. J. KNIAZEFF. 1973. Mechanism of demyelination in JHM virus encephalomyelitis. Electron microscopic studies. Acta Neuropathol. (Berl.) **24:** 76-85.
3. WEINER, L. P. 1973. Pathogenesis of demyelination induced by a mouse hepatitis virus. Arch. Neurol. **28:** 298-303.
4. POWELL, H. C. & P. W. LAMPERT. 1975. Oligodendrocytes and their myelin-plasma membrane connections in JHM mouse hepatitis virus encephalomyelitis. Lab. Invest. **33:** 440-445.
5. HERNDON, R. M., D. L. PRICE & L. P. WEINER. 1977. Regeneration of oligodendroglia during recovery from demyelinating disease. Science **195:** 693-694.
6. KNOBLER, R. L., M. V. HASPEL & M. B. A. OLDSTONE. 1981. Mouse hepatitis virus type 4 (JHM strain)-induced fatal central nervous system disease. I. Genetic control and the murine neuron as the susceptible site of disease. J. Exp. Med. **153:** 832-843.
7. RICHARDSON, E. P. 1961. Progressive multifocal leukoencephalopathy. N. Engl. J. Med. **265:** 815-823.
8. ZU RHEIN, G. M. & S. M. CHOU. 1965. Particles resembling papova viruses in human cerebral demyelinating disease. Science **148:** 1477-1479.
9. KRUPP, L. B., R. B. LIPTON, M. L. SWERDLOW, N. E. LEEDS & J. LLENA. 1985. Progressive multifocal leukoencephalopathy. Clinical and radiographic features. Ann. Neurol. **17:** 344-349.
10. AKSAMIT, A. J., P. MOURRAIN, J. L. SEVER & E. O. MAJOR. 1985. Progressive multifocal leokoencephalopathy. Investigation of three cases using in situ hybridization with JC virus biotinylated DNA probe. Ann. Neurol. **18:** 490-496.
11. LAMPERT, P. W. 1978. Autoimmune and virus-induced demyelinating diseases: A review. Am. J. Pathol. **91:** 176-208.
12. NAGASHIMA, K., H. WEGE, R. MEYERMANN & V. TER MEULEN. 1979. Demyelinating encephalomyelitis induced by a long-term corona virus infection in rats: A preliminary report. Acta. Neuropathol (Berl.) **45:** 205-213.
13. WATANABE, R., H. WEGE & V. TER MEULEN. 1983. Adoptive transfer of EAE-like lesions from rats with coronavirus-induced demyelinating encephalomyelitis. Nature **305:** 150-155.
14. THEILER, M. 1934. Spontaneous encephalomyelitis of mice: A new virus disease. Science **80:** 122.
15. LIPTON, H. L. 1975. Theiler's virus infection in mice: An unusual biphasic disease process leading to demyelination. Infect. Immunol. **11:** 1147-1155.
16. BARBANO, R. L. & M. C. DAL CANTO. 1984. Serum and cells from Theiler's virus-infected mice fail to injure myelinating cultures or to produce in vivo transfer of disease: The pathogenesis of Theiler's virus-induced demyelination appears to differ from that of EAE. J. Neurol. Sci. **66:** 283-293.

17. LAMPERT, P. W., R. S. FUJINAMI, M. RODRIGUEZ, J. L. LEIBOWITZ & W. LANG. 1983. Further studies on the pathogenesis of Theiler's murine encephalomyelitis (abstract). J. Neuropathol. Exp. Neurol. **42:** 326.
18. MILLER, S. D., R. J. CLATCH, D. C. PEVEAR, J. L. TROTTER & H. L. LIPTON. 1987. Class II-restricted T cell responses in Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease. I. Cross-specificity among TMEV substrains and related picornaviruses but not myelin proteins. J. Immunol. **138:** 3776-3784.
18a. RODRIGUEZ, M., C. F. LUCCHINETTI, R. J. CLARK, T. L. YAKSH, H. MARKOWITZ & V. A. LENNON. 1988. Immunoglobulins and complement in demyelination induced in mice by Theiler's virus. J. Immunol. **140:** 800-805.
19. MCCULLOUGH, B., S. KRAKOWKA & A. KOESTNER. 1974. Experimental canine distemper virus-induced demyelination. Lab. Invest. **31:** 216-222.
20. KRAKOWKA, S., B. MCCULLOUGH, A. KOESTNER & R. OLSEN. 1973. Myelin specific autoantibodies associated with central nervous system demyelination in canine distemper virus infection. Infect. Immunol. **8:** 819-827.
21. WISNIEWSKI, H. M. & B. R. BLOOM. 1975. Primary demyelination as a non-specific consequence of a cell-mediated reaction. J. Exp. Med. **141:** 346-359.
22. CAMMER, W., B. R. BLOOM, W. T. NORTON & S. GORDON. 1978. Degradation of basic protein in myelin by neutral proteases secreted by stimulated macrophages: A possible mechanism of inflammatory demyelination. Proc. Natl. Acad. Sci. USA **75:** 1554-1558.
23. POWELL, H. C., S. L. BRAHENY, R. A. C. HUGHES & P. W. LAMPERT. 1984. Antigen-specific demyelination and significance of the bystander effect in peripheral nerves. Am. J. Pathol. **114:** 443-453.
24. CLATCH, R. J., H. L. LIPTON & S. D. MILLER. 1986. Characterization of Theiler's murine encephalomyelitis virus (TMEV)-specific delayed type hypersensitivity responses in TMEV-induced demyelinating disease: Correlation with clinical signs. J. Immunol. **136:** 920-927.
25. DAL CANTO, M. C. & H. L. LIPTON. 1982. Ultrastructural immunohistochemical localization of virus in acute and chronic demyelinating Theiler's virus infection. Am. J. Pathol. **106:** 20-29.
26. LIPTON, H. L. & R. MELVOLD. 1984. Genetic analysis of susceptibility to Theiler's virus-induced demyelinating disease in mice. J. Immunol. **132:** 1821-1825.
27. CLATCH, R. J., R. W. MELVOLD, S. D. MILLER & H. L. LIPTON. 1985. Theiler's murine encephalomyelitis virus (TMEV) induced demyelinating disease in mice is influenced by the H-2D region: Correlation with TMEV-specific delayed-type hypersensitivity. J. Immunol. **135:** 1408-1414.
28. RODRIGUEZ, M. & C. S. DAVID. 1985. Demyelination induced by Theiler's virus: Influence of the H-2 haplotype. J. Immunol. **135:** 2145-2148.
29. RODRIGUEZ, M., J. LEIBOWITZ & C. S. DAVID. 1986. Susceptibility to Theiler's virus-induced demyelination: Mapping of the gene within the H-2D region. J. Exp. Med. **163:** 620-631.
30. RODRIGUEZ, M. & J. QUDDUS. 1986. Effect of cyclosporin A, silica quartz dust and protease inhibitors on virus-induced demyelination. J. Neuroimmunol. **13:** 159-174.
31. ROOS, R. P. & R. WOLLMANN. 1984. DA strain of Theiler's murine encephalomyelitis virus induces demyelination in nude mice. Ann. Neurol. **15:** 494-499.
32. ROSENTHAL, A., R. S. FUJINAMI & P. W. LAMPERT. 1986. Mechanism of Theiler's virus-induced demyelination in nude mice. Lab. Invest. **54:** 515-522.
33. RODRIGUEZ, M. & S. SRIRAM. 1987. Treatment of TMEV-induced demyelination with monoclonal antibodies to T cell subsets (abstract). Neurology **37**(Suppl. 1): 344.
34. LAMPERT, P. W. & M. RODRIGUEZ. 1984. Virus-induced demyelination. *In* Viral Pathogenesis, Vol. 1: 260-268. A. L. Notkins & M. B. A. Oldstone, Eds. Springer-Verlag. New York.
35. NITAYAPHAN, S., M. M. TOTH & R. P. ROOS. 1985. Localization of a neutralization site of Theiler's murine encephalomyelitis viruses. J. Virol. **56:** 887-895.
36. NITAYAPHAN, S., M. M. TOTH & R. P. ROOS. 1985. Neutralizing monoclonal antibodies to Theiler's murine encephalomyelitis viruses. J. Virol. **53:** 651-657.
37. SIGURDSSON, B. 1954. Rida, a chronic encephalitis of sheep. Br. Vet. J. **110:** 341-354.

38. PETURSSON, G., N. NATHANSON, G. GEORGSSON, H. PANITCH & P. A. PALSSON. 1976. Pathogenesis of visna. I. Sequential virologic, serologic, and pathologic studies. Lab. Invest. **35:** 402-412.
39. NARAYAN, O., D. E. GRIFFIN & J. CHASE. 1977. Antigenic drift of visna virus in persistently infected sheep. Science **197:** 376-378.
40. RODRIGUEZ, M., L. R. PEASE & C. S. DAVID. 1986. Immune-mediated injury of virus-infected oligodendrocyte: A model of multiple sclerosis. Immunol. Today **7:** 359-363.
41. LAMPERT, P. W., B. S. JOSEPH & M. B. A. OLDSTONE. 1976. Morphological changes of cells infected with measles or related to viruses. *In* Progress in Neuropathology, Vol. 3: 51-68. H. M. Zimmerman, Ed. Grune and Stratton. New York.
42. DAL CANTO, M. C. & H. L. LIPTON. 1975. Primary demyelination in Theiler's virus infection. An ultrastructural study. Lab. Invest. **33:** 626-637.
43. LINDSLEY, M. D. & M. RODRIGUEZ. 1987. Isolation of inflammatory cells from the CNS of mice persistently infected with Theiler's virus (abstract). J. Neuropathol. Exp. Neurol. **46:** 369.
44. LIPTON, H. L. & M. C. DAL CANTO. 1976. Theiler's virus-induced demyelination: Prevention by immunosuppression. Science **192:** 62-64.
45. ROOS, R. P., S. FIRESTONE, R. WOLLMAN, P. VARIAKOJIS & B. G. W. ARNASON. 1982. The effect of short-term and chronic immunosuppression on Theiler's virus demyelination. J. Neuroimmunol. **2:** 223-234.
46. RODRIGUEZ, M., W. P. LAFUSE, J. LEIBOWITZ & C. S. DAVID. 1986. Partial suppression of Theiler's virus-induced demyelination in vivo by administration of monoclonal antibodies to immune response gene products (Ia antigens). Neurology **36:** 964-970.
47. FRIEDMAN, A., G. FRANKEL, Y. LORCH & L. STEINMAN. 1987. Monoclonal anti-I-A antibody reverses chronic paralysis and demyelination in Theiler's virus-infected mice: Critical importance of timing of treatment. J. Virol. **61:** 898-903.
48. RODRIGUEZ, M., M. L. PIERCE & E. A. HOWIE. 1987. Immune response gene products (Ia antigens) on glial and endothelial cells in virus-induced demyelination. J. Immunol. **138:** 3438-3442.
49. RODRIGUEZ, M., J. L. LEIBOWITZ & P. W. LAMPERT. 1983. Persistent infection of oligodendrocytes in Theiler's virus-induced encephalomyelitis. Ann. Neurol. **13:** 426-433.
50. RODRIGUEZ, M. 1985. Virus-induced demyelination in mice: "Dying back" of oligodendrocytes. Mayo Clin. Proc. **60:** 433-438.
51. PENNEY, J. B., JR. & J. S. WOLINSKY. 1979. Neuronal and oligodendroglial infection by the WW strain of Theiler's virus. Lab. Invest. **40:** 324-330.
52. WROBLEWSKA, Z., S. U. KIM, W. D. SHEFFIELD & D. H. GILDEN. 1979. Growth of the WW strain of Theiler's virus in mouse central nervous system organotypic culture. Acta. Neuropathol. (Berl.) **47:** 13-19.
53. GRAVES, M. C., L. BOLOGA, L. SIEGEL & H. LONDE. 1986. Theiler's virus in brain cell cultures: Lysis of neurons and oligodendrocytes and persistence in astrocytes and macrophages. J. Neurosci. Res. **15:** 491-501.
54. RODRIGUEZ, M., D. HOVANEC-BURNS, L. SIEGEL & M. GRAVES. 1986. Cultured glial cells express Theiler's virus-associated antigen on their surfaces (abstract). Ann. Neurol. **20:** 143.
55. BRAHIC, M., W. G. STROOP & J. R. BARINGER. 1981. Theiler's virus persists in glial cells during demyelinating disease. Cell **26:** 123-128.
56. CHAMORRO, M., C. AUBERT & M. BRAHIC. 1986. Demyelinating lesions due to Theiler's virus are associated with ongoing central nervous system infection. J. Virol. **57:** 992-997.
57. AUBERT, C., M. CHAMORRO & M. BRAHIC. 1987. Identification of Theiler's virus infected cells in the central nervous system of mice during demyelinating disease. Microbiol. Pathogen. **31:** 319-326.
58. LUDWIN, S. K. 1981. Pathology of demyelination and remyelination. *In* Waxman, S. G. & Ritchie, J. M., Eds. Demyelinating Diseases: Basic and Clinical Electrophysiology. Advances in Neurology, Vol. 31: 123-168. Raven Press, New York.
59. LANG, W., M. RODRIGUEZ, V. A. LENNON & P. W. LAMPERT. 1984. Demyelination and remyelination in murine viral encephalomyelitis. Ann. N. Y. Acad. Sci. **436:** 98-102.
60. LUDWIN, S. K. 1978. Central nervous system demyelination and remyelination in the mouse. An ultrastructural study of cuprizone toxicity. Lab. Invest. **39:** 597-612.

61. DAL CANTO, M. C. & R. L. BARBANO. 1984. Remyelination during remission in Theiler's virus infection. Am. J. Pathol. **116:** 30-45.
62. RAINE, C. S. & U. TRAUGOTT. 1983. Chronic relapsing experimental autoimmune encephalomyelitis: Ultrastructure of the central nervous system of animals treated with combinations of myelin components. Lab. Invest. **48:** 275-284.
63. LANG, W., C. WILEY & P. LAMPERT. 1985. Theiler's virus encephalomyelitis is unaffected by treatment with myelin components. J. Neuroimmunol. **9:** 109-113.
64. RODRIGUEZ, M., V. A. LENNON, E. N. BENVENISTE & J. E. MERRILL. 1987. Remyelination by oligodendrocytes stimulated by antiserum to spinal cord. J. Neuropathol. Exp. Neurol. **46:** 84-95.