Murine Central Nervous System Infection by a Viral Temperature-Sensitive Mutant

A Subacute Disease Leading to Demyelination

MAURO C. DAL CANTO, MD and STANLEY G. RABINOWITZ, MD

From the Departments of Pathology (Neuropathology) and Medicine Northwestern University Medical School, Chicago, Illinois

Temperature-sensitive (*ts*) mutants of viruses may represent an important mechanism for viral persistence. *Ts* mutants of different complementation groups of vesicular stomatitus virus (VSV) have shown various disease patterns in infected mice which were at variance with the clinical and pathologic features of wild-type virus infection. To investigate whether neurovirulence of different *ts* mutants was dependent on the individual mutant or on the biochemical defect(s) common to all members of a complementation group, we infected mice with *ts* G32 VSV, a mutant of the same complementation group III as the previously described *ts* G31 VSV. Pathologic changes in infected mice were sharply different from those produced by *ts* G31 VSV and actually similar to those produced by *ts* G41 VSV, a member of Complementation Group IV, also previously described. These results suggest that the biologic behavior of *ts* mutants is dependent on the individual characteristics of each mutant. The most important alterations by *ts* G32 VSV were in the white matter of brain and spinal cord, where extensive inflammatory demyelination was observed. Lack of inflammation and demyelination in similarly infected nude mice would suggest that, in this infection, demyelination is produced by the host immune response rather than by direct viral myelinolytic activity. Such findings are similar to those we described in other viral infections and support the hypothesis of a common host-mediated pathway leading to demyelination in a variety of unrelated viral infections. These conclusions may have relevance to human demyelinating diseases. (Am J Pathol 1981, 102:412–426)

TEMPERATURE-SENSITIVE *(ts)* mutants of viruses appear to represent an important mechanism for the establishment and/or maintenance of persistent viral infections in a number of *in vitro* model systems.[1-14] On the other hand, attempts to define the role of *ts* viral mutants as etiologic agents in infections of animals and man are very limited. Among the more important studies, those involving *ts* mutants of reovirus in rats[15-17] and *ts* mutants of measles virus in hamsters[18,19] are especially notable for providing insights into the capacity of *ts* mutants to alter the pathogenesis of viral infections of the central nervous system (CNS). We have previously reported on murine models of vesicular stomatitis virus (VSV) CNS infection in which comparisons were made between the neurovirulence of parental, wild-type *(wt)* VSV and a number of *ts* VSV mutants.[20-24] Thus, whereas *wt* VSV invariably resulted in the rapid death of the mice, unaccompanied by substantial neuropathologic findings, certain *ts* mutants were associated with a marked attenuation of the infectious process and striking neuropathologic findings. For example, infection by *ts* G31 VSV (Complementation Group III) resulted in a peculiar spongiform encephalopathy, whereas *ts* G41 VSV (Complementation Group IV) produced a subacute infection accompanied by primary demyelination. Both patterns of disease were strikingly different from each other and from the *wt* virus infection.

Two important questions were raised by these studies. First, it was important to know whether different pathologic effects were dependent on specific biochemical defect(s) common to members of a particular complementation group or whether the pattern of disease was the property of an individual *ts* mutant regardless of the complementation group to which it belonged. Second, because the presence of primary de-

Supported by a grant from the Kroc Foundation and by Grants NS-13011 and NS-13045 from the National Institutes of Health.

Accepted for publication September 24, 1980.

Address reprint requests to Mauro C. Dal Canto, MD, Department of Pathology, Northwestern University Medical School, 303 East Chicago Avenue, Chicago, IL 60611.

0002-9440/81/0313-0412$01.25 © American Association of Pathologists

myelination in *ts* G41 infection was unexpected, we wondered whether such a pathologic process was a chance occurrence unique to one *ts* mutant or whether other mutants in other complementation groups were also capable of inducing demyelinating disease.

To help clarify these questions, we infected mice with a third *ts* mutant of VSV, *ts* G32, a member of VSV Complementation Group III, and compared CNS changes in such mice with those of animals infected with either *ts* G31, also a member of Complementation Group III, or with *ts* G41, a VSV Complementation Group IV mutant.

The results of this study suggest that pathologic alterations in the CNS of mice infected with *ts* mutants are dependent upon the individual biologic characteristics of each *ts* mutant and not on the biochemical mutation common to all members of a complementation group. The most surprising and interesting result in *ts* G32 infection was the presence of extensive demyelination in brain and spinal cord white matter, similar to, but more severe than in animals infected with *ts* G41 VSV. Moreover, experiments performed in nude mice, as well as results obtained from immunofluorescent studies, strongly suggest that demyelination in *ts* G32 infection is mediated through the host immune response rather than caused by direct cytolytic viral activity. Such results complement those in previously described viral demyelinating models[23,25-31] and support the possibility of a common mechanism of demyelination in a number of different viral infections.

Materials & Methods

Animals

Outbred Swiss-Webster mice, 3–4 weeks old, were purchased from Scientific Products (Arlington Heights, Ill). Balb/c mice and nude (nu/nu) and heterozygous nude (nu/ +) mice raised on a Balb/c background were obtained at 3–4 weeks of age from the Division of Mammalian Genetics, National Institutes of Health. All mice were housed in groups of 5–10 in polycarbonate cages and were provided with food and water *ad libitum*. Ambient room temperature was 21 C ± 1 C.

Infection With Virus

Mice were inoculated with 1.0×10^5 plaque-forming units (pfu) of VSV mutant *ts* G32 intracerebrally by the injection of 0.03 ml of inoculum through a 25-gauge needle while animals were maintained under light anesthesia. The viral suspension was diluted in Hanks' balanced salt solution.

Plaque Assay

BHK-21 cells were cultured in 6 well plates (35 × 10 mm, FB-6TC, Limbro Co., New Haven, Conn) in 2 ml of BHK-21 growth medium as previously described.[22] Plaque assays were performed with a conventional agar overlay containing neutral red as described previously.[22]

Preparation of Organs

The organs were obtained after exsanguination of the mice as previously described.[22]

Treatment of Tissues for Light-Microscopic and Ultrastructural Studies

Two to four animals were killed at 1–2-day intervals from 4 to 19 days postinfection (dpi). They were sacrificed by total body perfusion with chilled 3% glutaraldehyde in phosphate buffer (pH 7.4) under general anesthesia. Brains and spinal cords were removed and approximately 1-mm-thick sections were postfixed in osmic acid, dehydrated through a graded series of ethyl alcohol, cleared in propylene oxide, and embedded in Epon. Sections 1 μ thick were stained with toluidine blue and examined under the light microscope. Selected fields were trimmed for ultrathin sections, which were stained with uranyl acetate and lead citrate and viewed in a Philips 200 electron microscope.

Fluorescent Antibody Studies

The brains and spinal cords were quick-frozen and cut in a cryostat at a thickness of 6 μ. Sections were air-dried for 30 minutes, fixed in acetone for 10 minutes at room temperature, and then processed by an indirect immunofluorescent method employing rabbit antiserum to VSV (titer 1:20) and a sheep antiserum to rabbit globulin conjugated to fluorescein isothiocyanate (Grand Island Biological Co., Grand Island, NY). Hyperimmune antiserum to VSV was prepared in rabbits as previously described[24] and had a neutralizing antibody titer of 1:64:000. Controls for assessing the specificity of the reaction have also been described previously.[24]

The slides were viewed on a Zeiss standard microscope model IV (Carl Zeiss, New York, NY) with epi-illumination. Ultraviolet light was emitted by an HBO 50 LP 455 with barrier filters KP 540 and LP 520.

Results

Virologic Studies

Ts G32 VSV replicated both within the CNS and extraneurally (Figure 1). Titers of *ts* G32 in brain were

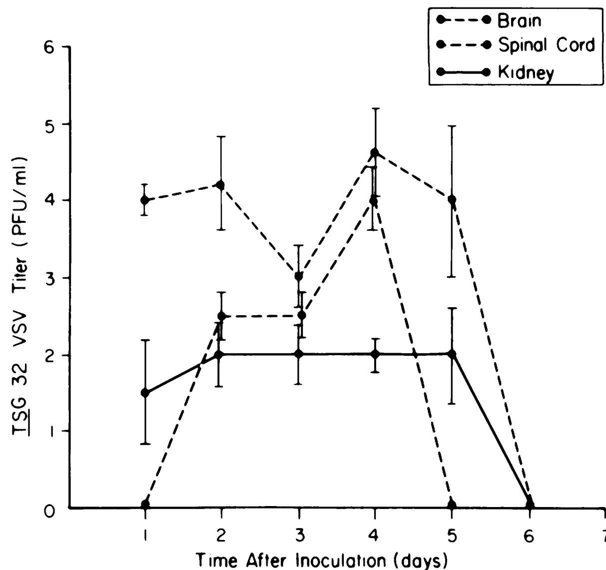


Figure 1—Recovery of temperature-sensitive (*ts*) vesicular stomatitis virus (VSV) G32 from brain, spinal cord, and kidneys of infected 3-4-week-old Swiss mice. *Ts* G32 (1×10^6 pfu) was inoculated intracerebrally, and the mice were sacrificed at various times after infection. Each point represents the arithmetic mean of 3 individual organ titrations assayed at 31 C in BHK-21 monolayers ± 1 SE.

about 10^4 PFU/ml, while spinal cord titers ranged between 10^2–10^4 PFU/ml. Virus was recovered from the CNS for 5 days after inoculation. Interestingly, *ts* G32 VSV was not recovered from either serum or spleens. In contrast to *ts* G32 VSV infection in Swiss mice, in nu/nu mice virus was recovered as long as 12 dpi, although the peak virus titers were similar.

Clinical Disease

Forty to fifty percent of Swiss, Balb/c, and heterozygous nude (nu/+) mice became sick around 5 dpi. They showed a loss in weight, ruffled fur, hunched back, and lethargy. They died around 10–12 dpi, at which time most showed paralysis of their hind legs. About 15–20% showed less severe generalized signs, developed hind-limb paralysis at 9–10 dpi, and survived in a paralyzed state until sacrificed.

Pathologic Findings

The following description refers to Swiss, Balb/c, and heterozygous (nu/+) mice.

Light Microscopy

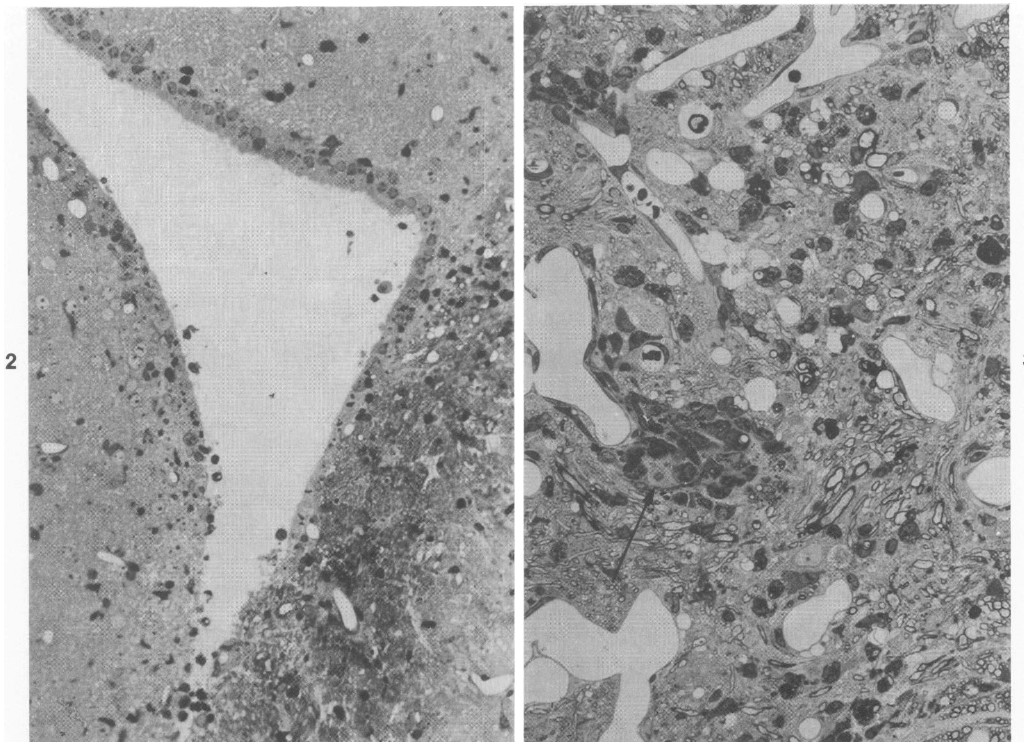
Brain Gray Matter: Starting at 6 dpi, small foci of parenchymal inflammatory infiltrates were observed. These were mainly localized around small vessels of gray matter, in hippocampi, and in periventricular areas of the cerebrum. Both lymphocytes and cells with larger round or folded nuclei characteristic of monocytes were present. The ependymal lining of the lateral ventricles appeared disrupted where inflammatory cells had reached the ventricular surface (Figure 2). During the next several days inflammatory infiltrates increased slightly, but some changes in the appearance and distribution of the cells were noted. Most of the larger mononuclear cells now showed numerous granular intracytoplasmic inclusions indicating they had converted to macrophages. Some plasma cells were recognizable in infected brain as early as 6 dpi, but they were more numerous at later time points.

Spinal Cord Gray Matter: The anterior horns were the first areas to show inflammatory infiltrates at about 6 dpi. As in the brain, these infiltrates were composed of lymphoid and larger mononuclear cells which seemed to gradually spread from a perivascular location into the surrounding neuropil. With time, these infiltrates became more severe and involved the central periependymal areas and often the posterior horns as well. Also, with time, large mononuclear cells became the dominant cell type and began to show intracytoplasmic granules and vacuoles, indicating transformation to macrophages. Where these infiltrates were most numerous, as in the anterior horns, normal structures were hard to recognize among the invading cells, and occasionally neuronal necrosis and neuronophagia were seen (Figure 3). During the third week of infection, the neuropil began to show reduced numbers of infiltrating cells, which now migrated toward blood vessels and formed rather thick perivascular collections. Ependymal cells were not affected. The meninges showed mononuclear infiltrates with cellular composition similar to that of the spinal cord parenchyma.

White Matter Changes in Brain and Spinal Cord: The most interesting finding was the presence, in white matter of brain and spinal cord, of focal areas of inflammatory cell infiltration accompanied by primary demyelination, beginning 9 dpi. In the brain these areas were exclusively localized to the corpus callosum (Figure 4), while in the spinal cord they were found in both anterior and lateral columns (Figure 5). In both brain and spinal cord most of infiltrating cells contiguous with demyelinated axons were lymphocytes, large monocytes, and macrophages. In the spinal cord these cells appeared as if they had spread from adjacent affected gray matter. Demyelinated axons showed normal axoplasm.

Electron Microscopy

Brain: A search for morphologic evidence of the presence of virus was attempted throughout the course of the study. Particular attention was given to



Figures 2-5 are of 1-μ-thick Epon-embedded sections of CNS from Balb/c or heterozygous nude (nu/+) mice infected with *ts* G32 VSV. The sections were stained with toluidine blue. **Figure 2**—Sub-ependymal inflammation is accompanied by focal destruction of ventricular ependyma in a Balb/c mouse, 7 dpi (×180) **Figure 3**—A nodule formed by mononuclear cells is present in the act of "neuronophagia." The *arrow* points to the sequestered neuron. Balb/c mouse, 11 dpi. (×280)

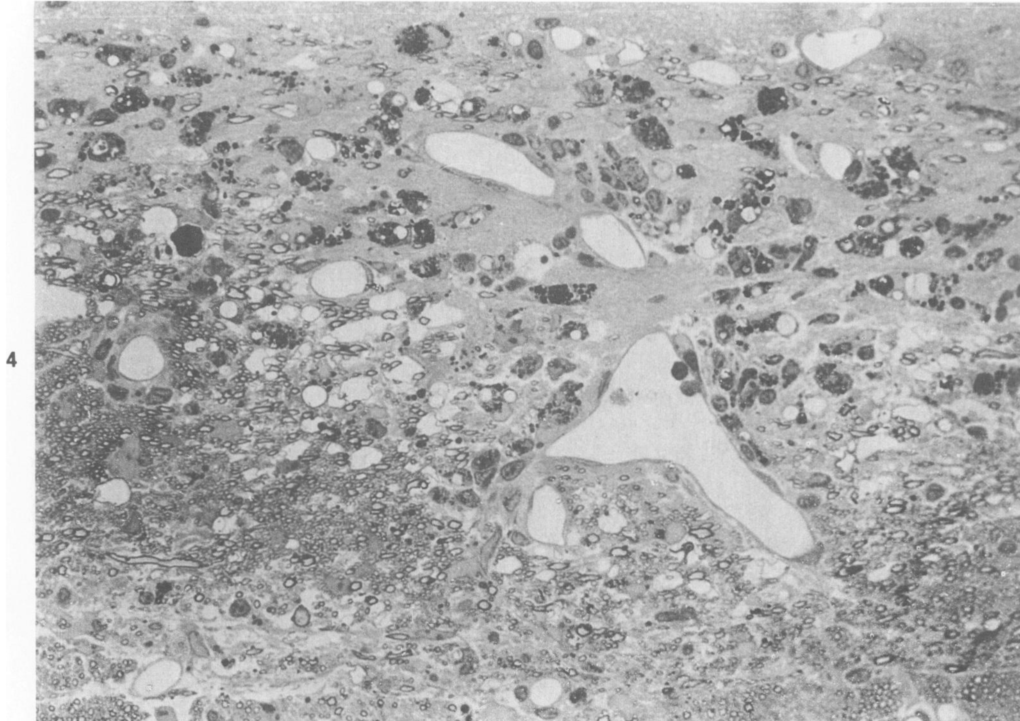
areas with inflammatory infiltrates, especially during the first week of infection, when viral titers were at their peak. No viral particles nor inclusions of viral matrix could be identified.

Lesions in the brain were very mild throughout the study. The most common findings consisted of focal alterations in the ependyma of the lateral ventricles. Such changes were found usually in the presence of inflammatory cells. The ependymal changes ranged from loss of cilia and microvilli in areas at some distance from the inflammatory cells to frank necrosis and disintegration where inflammatory infiltrates were more prominent (Figure 6). In denuded areas inflammatory cells abutted directly on the ventricular lumen, but they were also seen at some distance from the ventricular surfaces. Infiltrates mainly consisted of lymphocytes, plasma cells, and cells having irregularly shaped, indented nuclei and rather abundant cytoplasm. In areas of parenchymal disruption such cells transformed into macrophages and appeared laden with lamellar inclusions and dense bodies (Figure 7).

Spinal Cord Gray Matter: Lesions in the spinal cord gray matter were qualitatively similar to, although more severe than, those in the brain. At 9 dpi the anterior horns were infiltrated by mononuclear cells, some of which were recognizable as macrophages due to their abundant cytoplasmic debris (Figure 8). In the more heavily infiltrated areas scattered necrotic cells could be seen. Some could be recognized as neurons, but most had no distinguishing features. No viral particles nor viral matrix could be seen in any of the cells. At later time points increase in the extracellular space and a relative increase in astrocytic processes were noted.

Brain and Spinal Cord White Matter: A few scattered degenerated axons were seen in both brain and spinal cord white matter, especially in sections where maximal inflammation of gray matter was observed. The most striking changes, however, were those of primary myelin degeneration in the corpus callosum of the brain and, more extensively, in spinal cord white matter. The first changes were noticed around 9 dpi, 2 or 3 days after perivascular cuffs and inflammatory cells were first evident in the white matter. When fully developed, demyelination presented with morphologic patterns that were identical to those described in other experimental demyelinating diseases.[32,33] In some areas, mononuclear cells with various amounts of myelin debris were seen to surround

and enclose one or more axons with spiraling cytoplasmic processes. Such axons showed variable degrees of myelin loss, although no changes were apparent in their axoplasm (Figures 9–11). In other areas, myelin lamellas showed extensive vesicular disruption, which reduced them to networks of membranous arrays surrounding naked axons (Figure 12). One or more macrophages were always present nearby. These two patterns of primary myelin degeneration could also be seen in the same area, side by side.



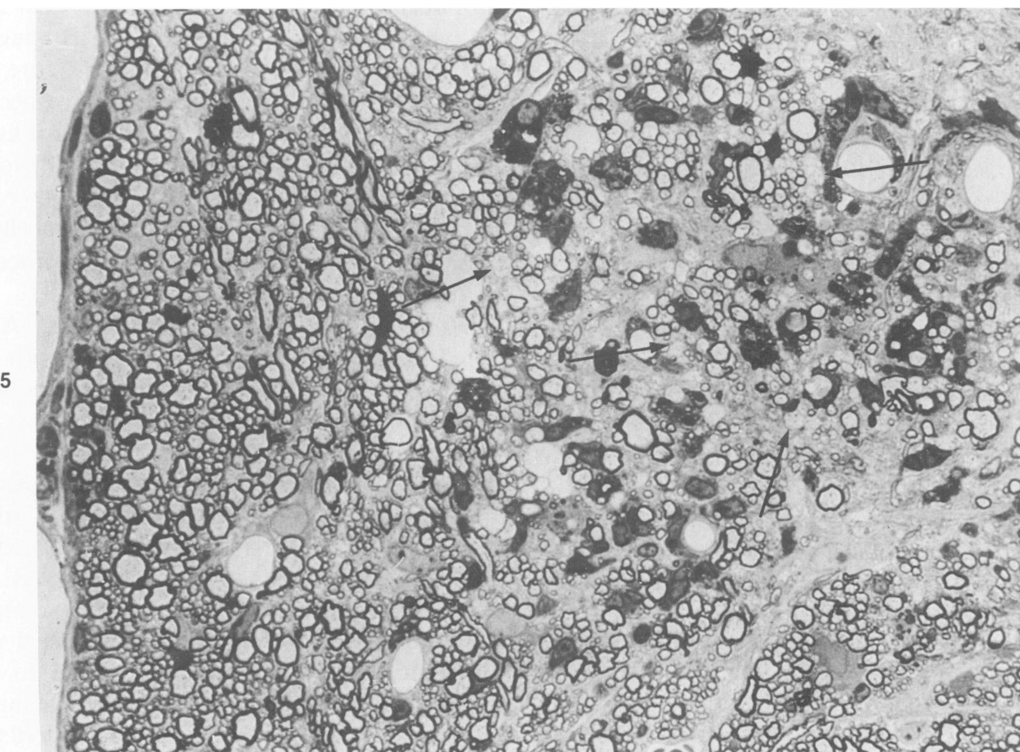
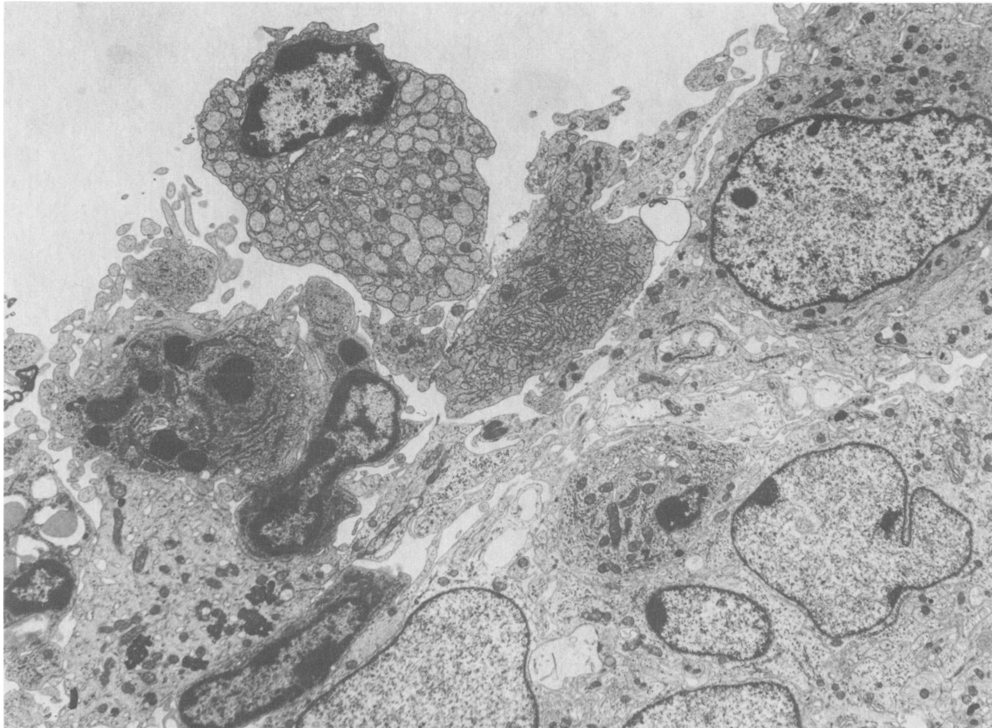
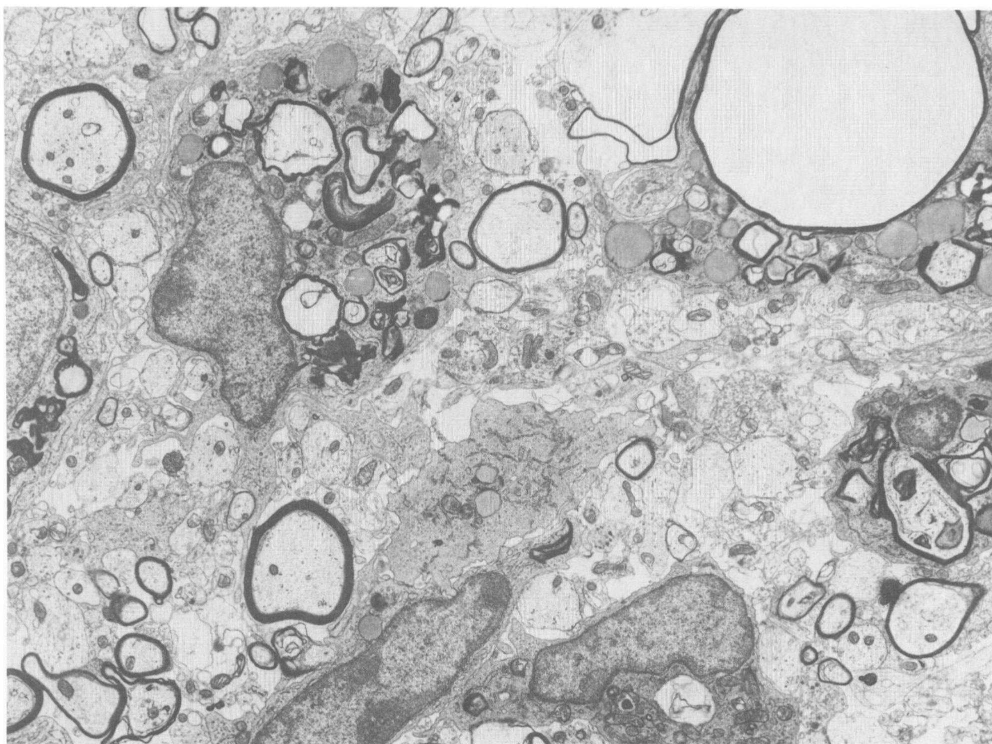


Figure 4—A large, well-circumscribed area of inflammation and demyelination is observed in the corpus callosum of a Balb/c mouse 10 dpi. (×280) **Figure 5**—A well-circumscribed area showing inflammation and numerous demyelinated axons is present in the lateral column of the spinal cord of a heterozygous nude (nu/+) mouse 11 dpi. (×420). The *arrows* point to some of the denuded axons.

Clinical and Pathologic Findings in Nude (nu/nu) Mice

Homozygous nude (nu/nu) mice injected with the same concentrations of *ts* G32 VSV as Swiss, Balb/c, and heterozygous nude (nu/ +) mice showed sharply different clinical and pathologic features. Clinically, most animals showed only mild weight loss, while about 5-10% became severely affected by a generalized illness without focal neurologic findings.





Figures 6-13 are electron micrographs from the CNS of Balb/c or heterozygous nude (nu/ +) mice infected with *ts* G32 VSV. The sections were stained with uranyl acetate and lead citrate. **Figure 6**—Inflammatory cells, including plasma cells, are seen on an altered ventricular surface. Brain of a Balb/c mouse 6 dpi. (× 5200) **Figure 7**—Mononuclear cells, including numerous debris-laden macrophages, are present in the brain parenchyma of a heterozygous nude (nude/ +) mouse 10 dpi. (× 7200)

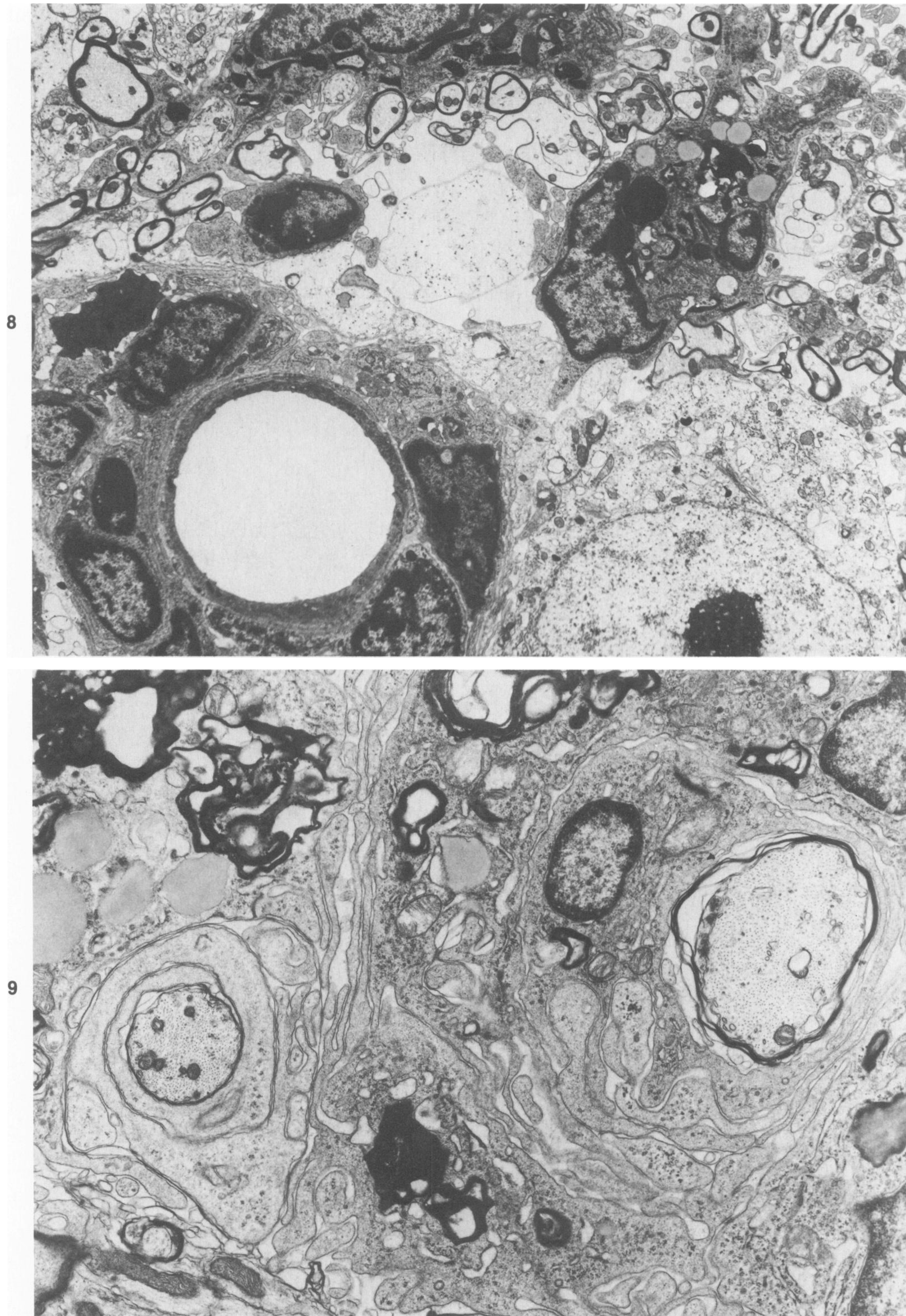
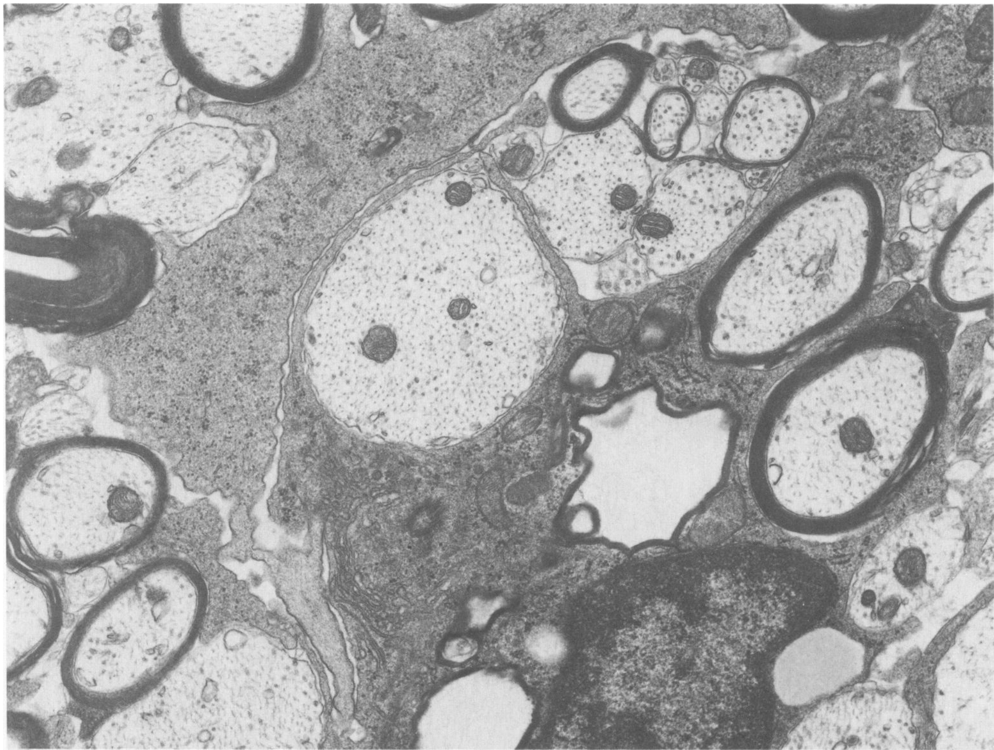
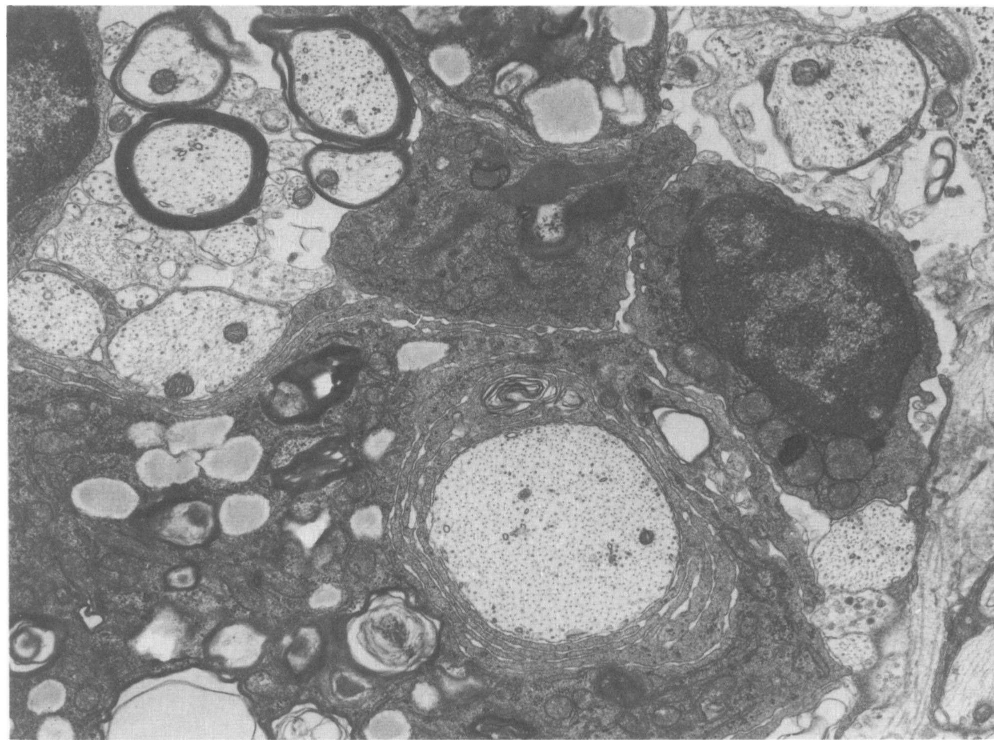


Figure 8—Mononuclear cells including macrophages are present in the ventral spinal horn of a heterozygous nude (nu/+) mouse 9 dpi. (×9200) **Figure 9**—Two axons are undergoing demyelination in the lateral column of the spinal cord of a Balb/c mouse 10 dpi. Note spiraling processes of mononuclear cells around axons which show an otherwise normal appearance. Abundant myelin debris is present. (×14,000)





Figures 10 and 11—Examples of demyelinated axons encircled by myelin debris-laden macrophages in the lateral columns of a heterozygous nude (nu/+) mouse 12 dpi. (**Figure 10**, ×19,800; **Figure 11**, ×15,900)

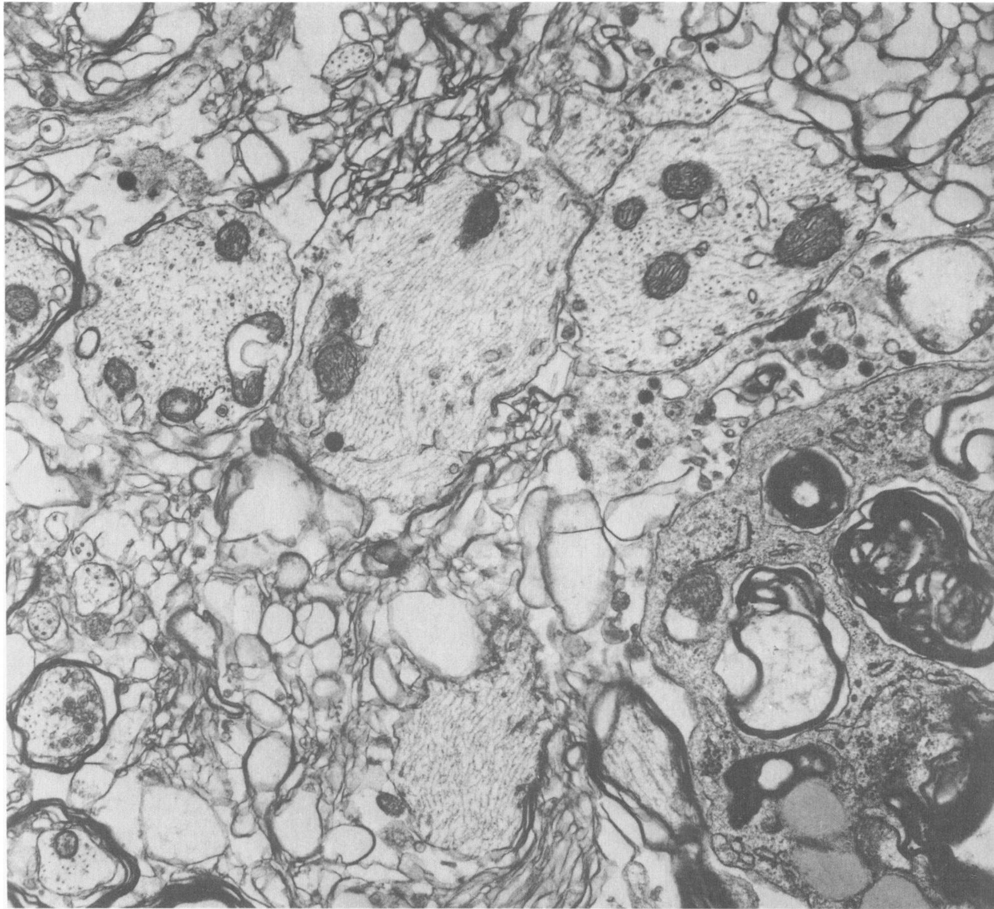


Figure 12—Numerous naked axons surrounded by myelin undergoing vesicular disruption are present in the anterior column of a Balb/c mouse 12 dpi. (×22,000)

Pathologically, the entire CNS appeared unremarkable except for small foci of gray matter necrosis accompanied by small numbers of inflammatory cells in the sickest animals. Special attention was given to the white matter of both brain and spinal cord. In parallel with the absence of infiltrating mononuclear cells, no evidence of demyelination was seen at either the light or ultrastructural level (Figure 13).

Immunofluorescence

Heterozygous Nude Mice

Viral antigen was present in gray matter cells, some of which were recognizable as neurons up to 5 dpi. Most cells, however, were not identifiable (Figure 14). From about 8 days, a few scattered cells with viral antigen were observed in the white matter of the spinal cord (Figure 15).

Nude Mice

Viral antigen was present in the gray matter of the spinal cord at 2 days and increased in the following days, reaching a peak at around 9 days. It was much more abundant than in heterozygous nude mice at the same time point and was distributed more extensively as fluorescent round cells were seen in the whole spinal cord gray matter (Figure 16). Interestingly, the spinal cord white matter of nude mice also contained more viral antigen than heterozygous mice, especially at later time points (Figure 17). Results indicating that more viral antigen is present in nude than in normal mice have also been reported in other virus infections.[34] This may be due to an impairment in virus clearance in athymic mice.

Discussion

Infection of immune-competent mice with *ts* G32 VSV produces an inflammatory disease of the CNS characterized by an acute to subacute course with hind-limb paralysis and both gray and white matter alterations. Most infected mice succumb around 10–12 days after infection, but about 10% survive and remain paralyzed.

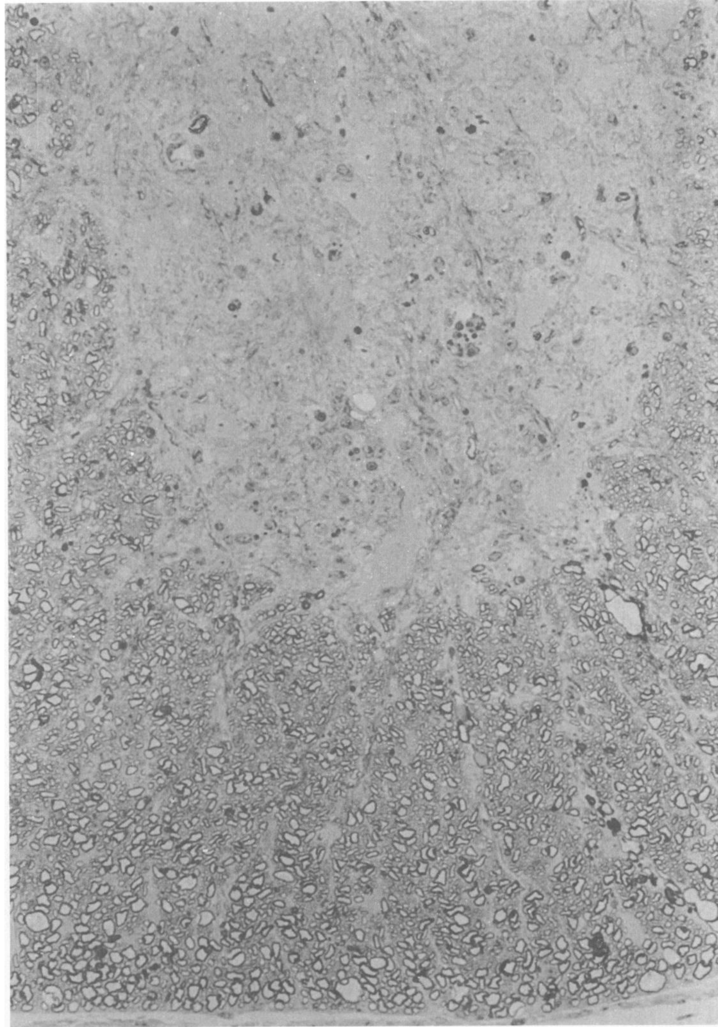
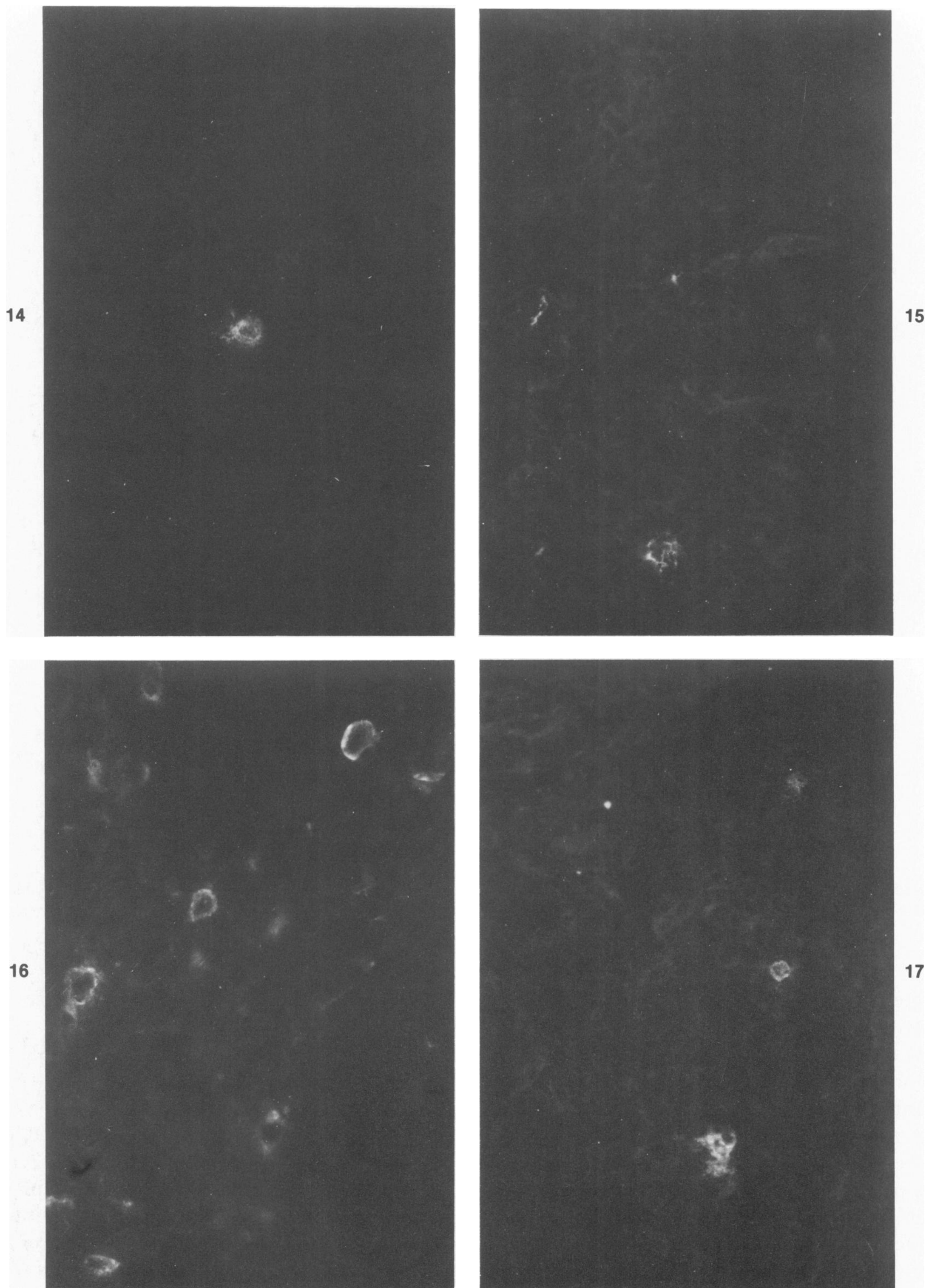


Figure 13—Epon-embedded section, 1 μ thick, of spinal cord from a nude (nu/nu) mouse 11 dpi shows mild inflammation of gray matter but no inflammation or demyelination of white matter. (Toluidine blue, ×150)

It is interesting to contrast *ts* G32 VSV infection with both *wt* VSV as well as other *ts* VSV mutants. Infection by *wt* VSV invariably led to death in 2-3 days with minimal pathologic changes in the CNS. Pathologic changes in *wt* VSV infection consisted of ependymal necrosis and large viral inclusions in anterior horn neurons.[24] Viral titers in brain and spinal cord reached a peak of 10^7 to 10^8 pfu/ml. *Ts* G31 (Complementation Group III) produced a slower CNS infection characterized by hind-limb paralysis and death in 6-9 days after inoculation. The pathologic hallmark of this infection was the presence of striking status spongiosus limited to the gray matter of the spinal cord. This spongiform myelopathy was mainly due to ballooning of dendrites and astrocytic processes with occasional involvement of neurons and myelin sheaths.[21] Viral titers in brain and spinal cord of mice infected with *ts* G31 were only 1% of those seen after *wt* VSV infection[35] (Tables 1 and 2).

Infection of Balb/c mice with *ts* G41 VSV (Complementation Group IV) produced the most protracted course of all the mutants we have tested.[22] Sixty percent of infected animals, in fact, developed hind-limb paralysis around 7 dpi, which lasted for 21-28 days. Although most mice recovered, 5-10% remained paralyzed for the whole period of observation, ie, 160 days. *Ts* G41 infection was pathologically characterized by the following features: 1) striking inflammation of spinal cord gray matter and leptomeninges lasting 5-6 weeks; 2) neuronal dropout with reactive astrocytosis; and 3) evidence of primary demyelination of the spinal cord in areas showing mononuclear inflammatory infiltrates.[23] Viral titers in Balb/c mice infected with *ts* G41 were only 1% of those seen after



Figures 14-17 show the presence of viral antigen by immunofluorescent staining of infected spinal cords. **Figure 14**—A positive cell is present in the ventral spinal horn of a heterozygous nude (nu/ +) mouse 4 dpi. (× 250) **Figure 15**—Irregular immunofluorescent profiles, probably cellular processes, are present in the spinal cord white matter of a heterozygous nude (nu/ +) mouse 10 dpi. (× 250) **Figure 16**—Numerous positive cells are present in the ventral spinal horn of a nude (nu/nu) mouse 7 dpi. (× 250) **Figure 17**—Three immunofluorescent cells are seen in the spinal cord white matter of a nude (nu/nu) mouse 12 dpi. (× 250)

Table 1—Comparison of Main Pathologic Changes in Three Different *ts* Mutants and Wild-Type VSV

	Complementation group	Inflammation of white matter	Demyelination of CNS white matter	Viral inclusions	Status spongiosus of gray matter
ts G32	III	yes	yes	no	no
ts G31	III	no	no	no	yes
ts G41	IV	yes	yes	no	no
wt VSV	—	no	no	yes	no

ts G31 infection. It is important to note that *ts* G41 could be recovered from both brain and spinal cord for up to 3 weeks after infection, and the rescued virus was exquisitely temperature-sensitive[22] (Tables 1 and 2).

Contrasting the present study with the other *ts* VSV infections is useful. First, the course of disease in animals infected with *ts* G32 approximates that of animals inoculated with *ts* G31, although a small percentage of mice infected with *ts* G32 had a more protracted course, lasting up to 19 days. Pathologically, however, there were no similarities. On the contrary, pathologic alterations in mice infected with *ts* G32 VSV were more similar to those observed in *ts* G41 infection. Viral titers in *ts* G32 infection were also similar to those observed after *ts* G41 inoculation (Tables 1 and 2).

It appears, therefore, that virus-CNS interactions after infection with different temperature-sensitive mutants are not directly related to a specific biochemical defect common to all mutants in a complementation group. Thus, viruses of Complementation Group III like *ts* G31 and *ts* G32 produced completely different neuropathologic alterations. In fact, *ts* G32 VSV (Complementation Group III) produced lesions that best approximated those produced by *ts* G41, a mutant of Complementation Group IV. One may conclude, therefore, that neuropathologic changes produced by different *ts* mutants may be more dependent upon the characteristics of each particular mutant rather than upon the biochemical alterations shared by all members of a complementation group.

Despite extensive search for evidence of viral maturation in affected areas of brain and spinal cord, no VSV virions could be observed at any time point in animals infected with *ts* G32 virus. This parallels our previous findings with *ts* G31 virus.[21] *Ts* mutants of Complementation Group III have been associated with defective synthesis and/or maturation of the M protein at nonpermissive temperatures.[36-38] M protein has an important role in viral maturation: it is responsible for the binding of the ribonucleoprotein material to the viral envelope.[39] In support of this notion, ultrastructural immunohistochemical studies from this laboratory have previously shown that spinal cord neurons of animals infected with *ts* G31 developed diffuse cytoplasmic accumulations of viral antigen, which contrasted with a discrete and localized distribution of subplasmalemmal antigen preceding viral budding in neurons infected with *wt* VSV.[40] Such findings indicated that viral proteins in *ts* G31 infection accumulated in the cytoplasm because of lack of viral assembly at the level of the cell membrane. Accumulation of unassembled viral proteins in the cytoplasm was also demonstrated by biochemical studies in infected neuroblastoma cells.[41] A similar situation is most probably occurring in cells infected with *ts* G32 virus in which lack of M protein maturation is postulated. The presence of viral antigens would still induce pathologic alterations, but no

Table 2—Virologic and Clinical Characteristics of Intracerebral Infection With Different *ts* Mutants and Wild-Type VSV

	Peak virus titer brain/spinal cord (pfu/ml)	Duration of virus recovery (days)	Rate of mortality	Mouse strain	Time to death (days)
ts G32	$10^4/10^2-10^4$	5	100	Swiss Balb/c nu/+	6-19
				nu/nu	10-20
ts G31	$10^6/10^5-10^6$	6-7	100	Swiss	5-9
ts G41	$10^4-10^5/10^2$	21	5-10	Balb/c	Sacrificed 21-28—otherwise most recovered
wt VSV	$10^8/10^7$	2-4	100	Swiss Balb/c	2-4

mature viral particles would be seen on routine ultrastructural examination.

Of special importance to us was the observation of extensive primary demyelination in brain and spinal cord white matter in mice infected with *ts* G32. The pathogenesis of demyelination in viral disease is of primary interest to our laboratory, and the present model exemplifies another viral infection in which demyelination is one of the major pathologic features.

Previous models reported by us include infections by Theiler's virus,[25,26] Chandipura virus,[27] and *ts* G41 VSV.[23] Theiler's virus and Chandipura virus infections have been studied most extensively with regard to the role of the immune response in the demyelinating process. By using immunosuppressive drugs in Theiler's virus infection[42] as well as studying nude mice infected with Chandipura virus,[27] we suggested that demyelination in these models may be mediated through the host immune response rather than being induced by direct viral cytolytic activity.

The present studies of *ts* G32 VSV in homozygous and heterozygous nude mice suggest that demyelination in this infection may depend on immunopathologic mechanisms similar to those in Theiler's and Chandipura virus infections. Where the heterozygous animals showed in fact inflammation and demyelination comparable to Swiss and Balb/c mice, the homozygous nude mice, which are severely impaired in their cell-mediated immune response, showed no evidence of myelin degeneration in the absence of inflammatory infiltrates. Immunofluorescent studies were also in support of these results. Lack of white matter alterations in nude mice contrasts in fact with the presence of viral antigen in the spinal cord of these animals. In fact, viral titers in nude mice persist longer than in outbred Swiss mice (Figure 18). This inverse relationship between the degree of virus antigen deposition and replication and absence of demyelination in the white matter of nude mice speaks against a mechanism of direct viral cytolysis and strongly favors an immunopathologic mechanism of demyelination. Thus heterozygous nude mice, fully capable of mounting an immune response, readily demonstrate demyelination even in the presence of scarce viral antigen, whereas their immune-compromised littermates, despite the presence of more viral antigen, are unable to produce the same pathologic changes.

Although *ts* G41 and *ts* G32 VSV possess the capacity to produce primary demyelination *in vivo*, it is not certain which properties of the virus are responsible for these histologic alterations. It is entirely conceivable that both *ts* G41 and *ts* G32 VSV are double mutants. Alternatively, the genes for *ts* and neurovirulence may be closely linked but separate. Nevertheless, it appears certain that the host's immune response is critical, once the virus has been introduced, in the expression of demyelination.

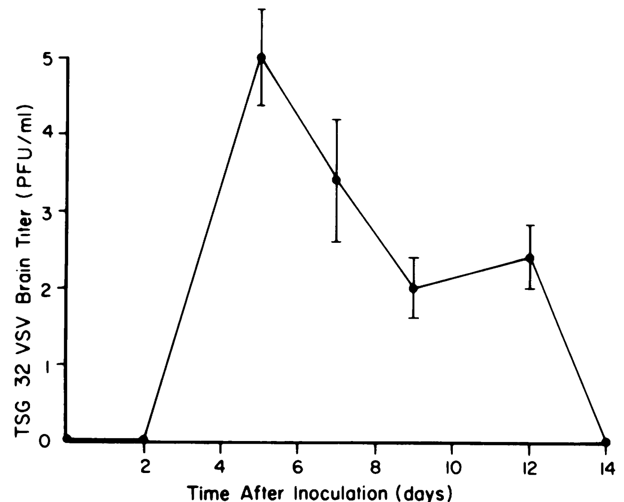


Figure 18—Recovery of temperature-sensitive (*ts*) vesicular stomatitis virus (VSV) G32 from brains of infected 3- to 4-week-old nu/nu mice. *Ts* G32 (1 × 10^6 pfu) was inoculated intracerebrally, and mice were sacrificed at various times after infection. Each point represents the arithmetic mean of 3 individual organ titrations assayed at 31 C in BHK-21 monolayers ± 1 SE.

Additional CNS models with a possible immune pathogenesis, described by others, are those of canine distemper virus infection[28-30] and Herpes simplex focal demyelination of the trigeminal root entry zone.[31] The possibility of a common mechanism of demyelination in a number of different viral infections is gaining strength as more examples are uncovered. As we[26,27,42] and others[43] previously suggested, the host antiviral immune response could represent the common denominator leading to demyelination in a number of viral diseases. In such cases myelin would be destroyed as an innocent "bystander" by the very immune response the host would mount against the virus. The bystander concept of myelin destruction finds roots in earlier studies by Ruddle and Waksman in a different system.[44] These authors demonstrated that rat embryo fibroblasts could be destroyed by lymphoid cells sensitized to an unrelated antigen in the presence of such antigen. Wisniewski and Bloom were the first to extend the concept of "bystander killing" to myelin in an *in vivo* system.[45] They showed primary demyelination in the spinal cord of guinea pigs sensitized to tuberculin, upon local CNS injection of such antigen. *In vitro* studies by other investigators also strongly support the hypothesis of bystander demyelination. For example, Cammer et al[46] showed that myelin basic protein could be degraded by neutral proteases and protease activators secreted by nonspecifically stimulated macrophages. Immune cells,

therefore, need not be specifically directed against myelin to cause myelin injury, but activation by unrelated antigen, including viruses, may be sufficient to arm them effectively against surrounding myelin. As more examples of virus-induced demyelination with similar pathogenesis are uncovered, the application of the bystander hypothesis of demyelination to viral infections gains support, and so does the relevance of models of virus-induced demyelination to human disease.

References

1. Henle G, Deinhardt F, Bergs VV, Henle W: Studies on persistent infections of tissue cultures: I. General aspects of the system. J Exp Med 1958, 108:537-560
2. Burge BW, Pfefferkorn ER: Isolation and characterization of conditional-lethal mutants of Sindbis virus. Virology 1966, 30:204-213
3. Cooper PD, Johnson RT, Garwes DJ: Physiological characterization of heat-defective (temperature-sensitive) poliovirus mutants: Preliminary classification. Virology 1966, 30:638-649
4. Simpson RW, Hirst GK: Temperature-sensitive mutants of influenza A virus: Isolation of mutants and preliminary observations of genetic recombination and complementation. Virology 1968, 35:41-49
5. Gharpure MA, Wright PF, Chanock RM: Temperature-sensitive mutants of respiratory syncytial virus. J Virol 1969, 3:414-421
6. Tan KB, Sambrook JF, Bellett AJD: Semliki Forest Virus temperature-sensitive mutants: Isolation and characterization. Virology 1969, 38:427-439
7. Pringle CR, Duncan IB: Preliminary physiological characterization of temperature-sensitive mutants of vesicular stomatitis virus. J Virol 1971, 8:56-61
8. Kimura G, Dulbecco R: A temperature-sensitive mutant of Simian virus 40 affecting transforming ability. Virology 1973, 52:529-534
9. Preble OT, Youngner JS: Selection of temperature-sensitive mutants during persistent infection: Role in maintenance of persistent Newcastle disease virus infections of L cells. J Virol 1973, 12:481-491
10. Preble OT, Youngner JS: Temperature-sensitive viruses and the etiology of chronic and inapparent infections. J Infect Dis 1975, 131:467-473
11. Rima BK, Martin SJ: Persistent infection of tissue culture cells by RNA viruses. Med Microbiol Immunol 1976, 162:89-118
12. Trousdale MD, Paque RE, Gauntt CJ: Isolation of coxsackievirus B3 temperature-sensitive mutants and their assignment to complementation groups. Biochem Biophys Res Commun 1977, 76:368-375
13. Hashimoto K, Suzuki K, Simizu B: Maturation defect of a temperature-sensitive mutant of western equine encephalitis virus. Arch Virol 1977, 53:209-219
14. Truant AL, Hallum JV: A persistent infection of baby hamster kidney—21 cells with mumps virus and the role of temperature-sensitive variants. J Med Virol 1977, 1:49-67
15. Fields BN, Raine CS, Baum SG: Temperature-sensitive mutants of reovirus type 3: Defects in viral maturation as studied by immunofluorescence and electron microscopy. Virology 1971, 43:569-578
16. Fields BN: Genetic manipulation of reovirus: A model for modification of disease? N Engl J Med 1972, 287:1026-1033
17. Raine CS, Fields BN: Neurotropic virus-host relationship alterations due to variation in viral genome as studied by electron microscopy. Am J Pathol 1974, 75:119-138
18. Haspel MV, Rapp F: Measle virus: An unwanted variant causing hydrocephalus. Science 1975, 187:450-451
19. Haspel MV, Duff R, Rapp F: Experimental measles encephalitis: a genetic analysis. Infect Immun 1975, 12:785-790
20. Rabinowitz SG, Dal Canto MC, Johnson TC: Comparison of central nervous system disease produced by wild-type and temperature-sensitive mutants of vesicular stomatitis virus. Infect Immun 1976, 13:1242-1249
21. Dal Canto MC, Rabinowitz SG, Johnson TC: An ultrastructural study of central nervous system disease produced by wild-type and temperature-sensitive mutants of vesicular stomatitis virus. Lab Invest 1976, 35:185-196
22. Rabinowitz SG, Johnson TC, Dal Canto MC: Subacute infection with temperature-sensitive vesicular stomatitis virus mutant G41 in the central nervous system of mice: I. Clinical and virologic studies. J Infect Dis 1979, 139:26-35
23. Dal Canto MC, Rabinowitz SG, Johnson TC: Subacute infection with temperature-sensitive vesicular stomatitis virus mutant G41 in the central nervous system of mice: II. Immunofluorescent, morphologic, and immunologic studies. J Infect Dis 1979, 139:36-51
24. Dal Canto MC, Rabinowitz SG, Johnson TC: *In vivo* assembly and maturation of vesicular stomatitis virus. Lab Invest 1976, 35:515-524
25. Lipton HL: Theiler's virus infection in mice: An unusual biphasic disease process leading to demyelination. Infect Immun 1975, 11:1147-1155
26. Dal Canto MC, Lipton HL: Primary demyelination in Theiler's virus infection: An ultrastructural study. Lab Invest 1975, 33:626-637
27. Dal Canto MC, Rabinowitz SG, Johnson TC: Virus-induced demyelination: Production by a viral temperature-sensitive mutant. J Neurol Sci 1979, 42:155-168
28. Wisniewski H, Raine CS, Kay WJ: Observations on viral demyelinating encephalomyelitis: Canine distemper. Lab Invest 1972, 26:589-599
29. McCullough B, Krakowka S, Koestner A: Experimental canine distemper virus-induced demyelination. Lab Invest 1974, 31:216-222
30. Raine CS: On the development of CNS lesions in natural canine distemper encephalomyelitis. J Neurol Sci 1976, 30:13-28
31. Townsend JJ, Baringer JR: Central nervous system susceptibility to Herpes simplex infection. J Neuropathol Exp Neurol 1978, 37:255-262
32. Alvord EC Jr: Acute disseminated encephalomyelitis and "allergic neuroencephalopathies," Handbook of Clinical Neurology. Vol 9. Edited by PJ Vinken, GW Bruyn. Amsterdam, North-Holland Publishing Co, 1970, pp 500-571
33. Lampert PW: Autoimmune and virus-induced demyelinating diseases. Am J Pathol 1978, 91:176-208
34. Wyde PR, Couch RB, Mackler BF, Cate TR, Levy BM: Effects of low- and high-passage influenza virus infection in normal and nude mice. Infect Immun 1977, 15:221-229
35. Rabinowitz SG, Johnson TC, Dal Canto MC: The uncoupled relationship between the temperature-sensitivity and neurovirulence in mice of mutants of vesicular stomatitis virus. J Gen Virol 1977, 35:237-249
36. Printz P, Wagner RR: Temperature-sensitive mutants of vesicular stomatitis virus: Synthesis of virus specific proteins. J Virol 1971, 7:651-662
37. Lafay F: Envelope proteins of vesicular stomatitis virus:

Effect of temperature-sensitive mutations in complementation groups III and V. J Virol 1974, 14:1220–1228
38. Knipe D, Lodish HF, Baltimore D: Analysis of the defects of temperature-sensitive mutants of vesicular stomatitis virus: Intracellular degradation of specific viral proteins. J Virol 1977, 21:1140–1148
39. Knipe DM, Baltimore D, Lodish HF: Maturation of viral proteins in cells infected with temperature-sensitive mutants of vesicular stomatitis virus. J Virol 1977, 21:1149–1158
40. Dal Canto MC, Rabinowitz SG, Johnson TC, Hughes JV: Ultrastructural-immunohistochemical evidence for a maturation defect of temperature-sensitive G31 vesicular stomatitis virus in murine spinal cord neurons. Infect Immun 1979, 24:276–281
41. Hughes JV, Johnson TC, Rabinowitz SG, Dal Canto MC: Growth and maturation of a vesicular stomatitis virus temperature-sensitive mutant and its central nervous system isolate. J Virol 1979, 29:312–321
42. Lipton HL, Dal Canto MC: Theiler's virus-induced demyelination: Prevention by immunosuppression. Science 1976, 192:62–64
43. Wisniewski HM: Immunopathology of demyelination in autoimmune diseases and virus infections. Br Med Bull 1977, 33:54–59
44. Ruddle NH, Waksman BH: Cytotoxicity mediated by soluble antigen and lymphocytes in delayed hypersensitivity: 1. Characterization of the phenomenon. J Exp Med 1968, 128:1237–1254
45. Wisniewski HM, Bloom BR: Primary demyelination as a nonspecific consequence of a cell-mediated immune reaction. J Exp Med 1975, 141:346–359
46. Cammer W, Bloom BR, Norton WT, Gordon S: Degradation of basic protein in myelin by neutral proteases secreted by stimulated macrophages: A possible mechanism of inflammatory demyelination. Proc Natl Acad Sci USA 1978, 75:1554–1558

Acknowledgments

The expert technical assistance of Jannie Tong, Jayashree Huprikar, and Mary Grover as well as the secretarial assistance of Lola Rivero are gratefully acknowledged.