Immunopathology and Infectious Disease

Contrasting Roles for Axonal Degeneration in an Autoimmune versus Viral Model of Multiple Sclerosis

When Can Axonal Injury Be Beneficial?

Ikuo Tsunoda, Tomoko Tanaka, Emily Jane Terry, and Robert S. Fujinami

From the Department of Neurology, University of Utah School of Medicine, Salt Lake City, Utah

Although demyelination is a cardinal feature in multiple sclerosis, axonal injury also occurs. We tested whether a delay in axonal degeneration could affect the disease severity in two models for multiple sclerosis: experimental autoimmune encephalomyelitis (EAE) and Theiler's murine encephalomyelitis virus (TMEV) infection. We compared wild-type C57BL/6 (B6) mice with C57BL/Wld^a (Wld) mice, which carry a mutation that delays axonal degeneration. In EAE, both mouse strains were sensitized with myelin oligodendrocyte glycoprotein (MOG)₃₅₋₅₅ peptide and showed a similar disease onset, MOG-specific lymphoproliferative responses, and inflammation during the acute stage of EAE. However, during the chronic stage, B6 mice continued to show paralysis with a greater extent of axonal damage, demyelination, and MOG-specific lymphoproliferative responses compared with Wld mice, which showed complete recovery. In TMEV infection, only Wld mice were paralyzed and had increased inflammation, virus antigen-positive cells, and TMEV-specific lymphoproliferative responses versus infected B6 mice. Because TMEV can use axons to disseminate in the brain, axonal degeneration in B6 mice might be a beneficial mechanism that limits the virus spread, whereas slow axonal degeneration in Wld mice could favor virus spread. Therefore, axonal degeneration plays contrasting roles (beneficial versus detrimental) depending on the initiator driving the disease. (*Am J Pathol* 2007, 170:214–226; DOI: 10.2353/ajpath.2007.060683)

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS). The etiology is believed to have both immune-mediated and en-

vironmental components, particularly virus infection.^{1–4} Experimental autoimmune (allergic) encephalomyelitis (EAE) and Theiler's murine encephalomyelitis virus (TMEV) infection have been used to study the possible autoimmune and viral mechanisms for MS.⁵ EAE can be induced in animals with a subcutaneous injection of CNS antigen emulsified with complete Freund's adjuvant or adoptive transfer of CNS antigen-specific CD4⁺ T cells. TMEV is a nonenveloped, positive-sense, single-stranded RNA virus, belonging to the genus *Cardiovirus*, family *Picornaviridae*.⁶ Intracerebral infection of TMEV causes an inflammatory demyelinating disease in the CNS in susceptible mouse strains. The two models are similar to MS clinically and histologically, in which cellular and humoral immune responses are believed to play important roles in the pathogenesis.

Pathological and neuroimaging studies in MS reported the presence of axonal degeneration, including Wallerian degeneration.^{7,8} Immunostaining studies have provided direct evidence of axonal damage using antibodies against two markers for damaged axons: nonphosphorylated neurofilament⁹ and amyloid precursor protein.¹⁰ This approach made possible the identification of earlier and subtler changes in axons than those seen by silver staining. Immunohistochemical studies demonstrated that axonal damage also occurs in animal models for MS, including EAE and TMEV infection.^{11,12} New imaging techniques have been used to evaluate axonal damage in MS. These techniques include measuring T1 hypointense lesions or T1 “black holes,” magnetization transfer imaging, magnetic resonance spectroscopy, and determining spinal and cerebral atrophy.¹³ Some studies have

Supported by the National Multiple Sclerosis Society (pilot grant PP0994 to I.T.), the University of Utah (funding incentive seed grant to I.T.), and the National Institutes of Health (grant NS34497 to R.S.F.).

Accepted for publication September 15, 2006.

Address reprint requests to Robert S. Fujinami, Ph.D., Department of Neurology, University of Utah School of Medicine, 30 North 1900 East, Room 3R330, Salt Lake City, UT 84132. E-mail: robert.fujinami@hsc.utah.edu.

shown that it is axonal loss and dysfunction rather than demyelination that is responsible for the subsequent disability observed in MS. In addition, although Wallerian degeneration in the CNS was thought to be a rare occurrence in MS, autopsy and magnetic resonance imaging studies have demonstrated Wallerian degeneration in patients with MS.^{14–17}

Important insight into the mechanisms of axonal degeneration came with findings in C57BL/Wld^s (C57/OlaHsd-Wld, Wallerian degeneration slow mutant) (Wld) mice, which are a substrain of C57BL/6 (B6) mice and have prolonged survival of the distal stumps of transected axons.^{18–20} Transected axons from Wld mice survive for up to 4 weeks, support action potentials for at least 2 weeks, and continue anterograde and retrograde transport of proteins for similar amounts of time.^{21–23} Genetic studies have attributed this slow Wallerian degeneration phenotype to the overexpression of a fusion protein (Wld^s) that is composed of the N-terminal 70 amino acids of ubiquitin fusion degradation protein 2a (Ufd2a), a ubiquitin-chain assembly factor, fused to the complete sequence of nicotinamide mononucleotide adenylyltransferase 1 (Nmna1), an enzyme in the nicotinamide adenine dinucleotide (NAD) biosynthesis pathway.²⁴ Both the ubiquitin proteasome system and Nmna1 have been shown to play an important role in axonal degeneration.²⁵ Wang and colleagues²⁶ demonstrated that NAD levels decreased in degenerating axons and that the exogenous application of NAD or its precursor, nicotinamide, prevented axonal degeneration.

Although preservation of axons seems to be beneficial for individuals, a lack or delay of axonal degeneration can be detrimental in some instances. For example, TMEV has been shown to spread in the CNS, via axonal flow. Thus, preservation of axons during TMEV infection should favor virus spread in the CNS. To test whether the delay of axonal degeneration could affect disease severity in an autoimmune and a viral model for MS, we induced EAE and TMEV infection in B6 mice and Wld mice. In EAE during the acute stage, both mouse strains showed a similar disease clinically, histologically, and immunologically. During the chronic stage, Wld mice had no clinical signs with a decline in myelin-specific T-cell responses. In contrast, B6 mice showed disease progression both clinically and histologically with high levels of myelin-specific T-cell lymphoproliferation. Interestingly, in TMEV infection, although B6 mice had no clinical disease, Wld mice developed paralysis. Wld mice had increased inflammation, virus antigen-positive cells, and TMEV-specific lymphoproliferative responses compared with B6 mice. Therefore, axonal degeneration can play contrasting roles (beneficial versus detrimental) depending on the type of disease.

Materials and Methods

EAE Induction and TMEV Infection

B6 and Wld mice were purchased from Harlan (Bicester, Oxon, UK). Female mice were sensitized subcutaneously

in the base of the tail with 100 nmol of myelin oligodendrocyte glycoprotein (MOG)_{35–55} peptide (MEVGWYR-SPFSRVVHLYRNGK) (Core Facility of the University of Utah Huntsman Cancer Institute, Salt Lake City, UT)²⁷ in complete Freund's adjuvant composed of Imject Freund's incomplete adjuvant (Pierce Biotechnology, Rockford, IL) and *Mycobacterium tuberculosis* H37 Ra (Difco Laboratories, Detroit, MI). The final concentration of *M. tuberculosis* in the MOG/complete Freund's adjuvant solution was 2 mg/ml (200 µl/mouse). The mice were injected intraperitoneally with 400 ng of pertussis toxin (List Biological Laboratories, Campbell, CA) in 200 µl of phosphate-buffered saline (PBS) on days 0 and 2 after MOG sensitization. The Daniels (DA) strain of TMEV was propagated in baby hamster kidney (BHK)-21 cells (American Type Culture Collection, Manassas, VA). Male mice were infected intracerebrally with 2×10^5 plaque-forming units of TMEV.

Histology

Mice were euthanized with isoflurane (IsoSol; Vedco Inc., St. Joseph, MO) either at weeks 2 and 3 and months 1 and 2 after MOG sensitization or at weeks 1, 2, 3, and 5 and months 3 and 6 after TMEV infection. We perfused mice with PBS, followed with a 4% paraformaldehyde solution (Sigma-Aldrich, St. Louis, MO). Brains were divided into five coronal slabs and spinal cords were divided into 10 to 12 transverse slabs. Muscles were harvested from lower extremities. Tissues were embedded in paraffin. Four-µm-thick CNS sections were stained with Luxol fast blue (Solvent Blue 38; Sigma-Aldrich) for myelin visualization. Muscle sections were stained with hematoxylin and eosin (H&E). The numbers of perivascular cuffs in all brain sections were counted as described previously.²⁸ Histological scoring was performed as previously described.²⁸ Brain sections were scored for meningitis (0, no meningitis; 1, mild cellular infiltrates; 2, moderate cellular infiltrates; 3, severe cellular infiltrates), perivascular cuffing (0, no cuffing; 1, 1 to 10 lesions; 2, 11 to 20 lesions; 3, 21 to 30 lesions; 4, 31 to 40 lesions; 5, more than 40 lesions), and demyelination (0, no demyelination; 1, mild demyelination; 2, moderate demyelination; 3, severe demyelination). Each score from the brain was combined for a maximum score of 11 per mouse. TMEV antigen and damaged axons were visualized by the avidin-biotinylated enzyme complex (ABC) technique, using hyperimmune rabbit serum to DA virus²⁹ and SMI 311, a cocktail of monoclonal antibodies (SMI 32, 33, 37, 38, and 39) to nonphosphorylated neurofilament with auto-clave pretreatment.^{9,12} Enumeration of TMEV antigen-positive cells was performed with a light microscope, at a magnification of $\times 200$, using five coronal brain sections and 10 to 12 transverse spinal cord sections per mouse as described previously.²⁸

CNS Viral Titers

Infected mice were euthanized and perfused with PBS at weeks 1, 2, 3, and 5 and months 3 and 6 after TMEV

infection. The brains and spinal cords were aseptically removed, weighed, and homogenized in PBS. The homogenates were frozen and thawed three times and plaqued on BHK-21 cell monolayers.^{28,30}

Lymphoproliferative Assays

Inguinal lymph nodes or spleens were removed and pooled from EAE mice or TMEV-infected mice, respectively, and mononuclear cells (MNCs) were isolated with Histopaque-1083 (Sigma-Aldrich). A volume of 100 μ l containing 2×10^5 cells in RPMI 1640 (Mediatech, Inc., Herndon, VA) supplemented with 1% glutamine (Mediatech), 1% antibiotics (Mediatech), 50 μ mol/L 2-mercaptoethanol (Sigma-Aldrich), and 10% fetal bovine serum (Invitrogen, Carlsbad, CA) was added to each well of 96-well plates. This was followed with 100 μ l of MOG₃₅₋₅₅ or bovine myelin basic protein (MBP) such that the final protein concentration was 50 μ g/ml in EAE experiments or with 100 μ l of solution containing 2×10^5 TMEV-antigen presenting cells (APCs) or live DA virus (multiplicity of infection = 5) in TMEV experiments. Purified MBP was prepared as described by Deibler and colleagues.³¹ TMEV-APCs were made from whole spleen cells infected *in vitro* with TMEV at an multiplicity of infection of 1 and irradiated with 2000 rads using a ¹³⁷Cs irradiator.³² For the antibody blocking experiments, CD4 (clone GK 1.5; American Type Culture Collection), CD8 (Lyt2.43; American Type Culture Collection), or major histocompatibility complex (MHC) class II (Y-3P; American Type Culture Collection)³³ antibody was added at culture initiation.³⁴ The cells were then cultured for 4 days at which time each well was pulsed with 1 μ Ci of tritiated thymidine (PerkinElmer Life Sciences, Boston, MA), and cells were cultured for another 24 hours. Cultures were harvested onto filters using a multiwell cell harvester (Molecular Devices, Sunnyvale, CA) and ³H incorporation was determined.³⁵ All cultures were performed in triplicate and some results were expressed as stimulation indexes (experimental cpm/control cpm).

Results

Clinical Course of MOG-Induced EAE

We induced EAE in B6 and Wld mice and compared clinical courses between the two strains. Among mice that had clinical signs, both mouse strains showed a similar disease onset and severity for the first 2 weeks after sensitization with MOG (mean disease onset day $\pm$ SEM: B6 mice, 14.2 ± 0.5 ; Wld mice, 13.5 ± 0.9 , $P > 0.05$ by *t*-test) (Figure 1). After this time point, however, B6 mice showed further disease progression until 3 weeks after sensitization. Approximately half of the B6 mice developed complete flaccid paralysis of both hind limbs, but incontinence was rare. During the chronic stage, B6 mice did not recover completely and had neurological sequelae. Relapse (second attack) was seen only in a few mice. In contrast, Wld mice showed only mild paral-

Figure 1. Clinical score of mice with EAE. C57BL/6 mice (B6, filled circle) and C57BL/Wld mice (Wld, open triangle) were sensitized with MOG₃₅₋₅₅ on day 0. Among mice that had clinical signs, both mouse strains showed a similar disease onset and progression until 2 weeks after sensitization. From this time point, B6 mice showed further disease progression with higher clinical scores than those of Wld mice (** $P < 0.01$, * $P < 0.05$; *t*-test). During the chronic stage, B6 had neurological deficit, whereas Wld mice recovered completely. Shown are mean clinical scores $\pm$ SEM of 17 B6 mice and 16 Wld mice with clinical signs. Results are representative of two independent experiments.

ysis with no disease progression and completely recovered. Wld mice had significantly lower clinical scores than those of B6 mice (mean clinical scores $\pm$ SEM on day 19: B6 mice, 3.2 ± 0.3 ; Wld mice, 0.9 ± 0.3 , $P < 0.01$ by *t*-test) (Figure 1).

Neuropathology of EAE

At disease onset, ~2 weeks after sensitization with MOG₃₅₋₅₅, both mouse strains had a similar distribution of lesions and severity. Initial cellular infiltrates composed both of MNCs and polymorphonuclear cells were present mainly in the meninges. With time, inflammation comprised of only MNCs with mild demyelination was seen in the anterior funiculus, ventral root exit zone, the gracile fasciculus of the posterior funiculus, and the conus medullaris of the spinal cord (Figure 2, a and b). In the brain, mild to moderate meningitis was present, and perivascular cuffing and demyelination were less severe than that found in the spinal cord. No difference was seen in brain pathology scores between the two mouse strains (Figure 2g). Three weeks after sensitization, B6 mice developed large inflammatory demyelinating lesions at all levels of the spinal cord. The demyelinating white matter had vacuolar changes, suggesting axonal degeneration. In contrast, Wld mice had only small inflammatory demyelination lesions in the subpial areas of only a few segments of the spinal cord (Figure 2, c and d).

One month after sensitization, B6 mice had smaller demyelinating lesions than those seen at 3 weeks, and some areas appeared as shadow-plaques, suggesting remyelination. Inflammation was seen in the meninges, corpus callosum, and cerebellar white matter in the brain

Figure 2. Neuropathology of MOG-induced EAE mice. **a** and **b**: On disease onset, both B6 (**a**) and Wld (**b**) mice develop a MNC infiltration in the meninges (**arrowheads**) with mild subpial demyelination along the anterior fissure of the spinal cord. **c** and **d**: Three weeks after sensitization, B6 mice (**c**) developed extensive perivascular cuffings (**arrow**) and vacuolar demyelination in the anterior funiculus of the spinal cord, whereas Wld mice (**d**) had meningeal (**arrowheads**), but not parenchymal, cell infiltration with mild demyelination. **e** and **f**: One month after sensitization, B6 mice (**e**) had inflammatory demyelinating lesions (**arrow**) in the corpus callosum, whereas Wld mice (**f**) had no lesions in the brain. **g**: The neuropathology scores were compared between B6 (closed column) and Wld (open column) mice based on the severity of meningitis, perivascular cuffing, and demyelination in the brain. Both strains had similar pathology scores at 2 weeks after sensitization. However, through 1 month after sensitization, B6 mice had sustained inflammatory demyelinating lesions, whereas the lesions in Wld mice subsided by 1 month after sensitization (** $P < 0.01$, *t*-test). At each time point, 5 to 12 mice were analyzed. Values are means $\pm$ SEM. Shown are representative results from two independent experiments. **a–f**: Luxol fast blue staining. Original magnifications, $\times 37$.

(Figure 2e), but inflammation was less prolonged in the spinal cord. Two months after sensitization, B6 mice had mild meningitis and perivascular cuffing in the brain. In the spinal cord, small areas of demyelination were often found in the subpial areas of the gracile fasciculus of the posterior funiculus. In contrast, at both 1 and 2 months after sensitization, approximately half of the Wld mice had no lesions in the CNS (Figure 2f), whereas the other half had only mild meningitis in the brain or small demyelinating lesions in the spinal cord. The brain pathology score was significantly lower in Wld mice than in B6 mice, 1 month after sensitization (Figure 2g; $P < 0.01$, *t*-test). We

Figure 3. Axonal degeneration in the spinal cord of EAE mice. We detected axonal damage (**arrow**) using immunohistochemistry against nonphosphorylated neurofilament. **a** and **b**: B6 mice had large numbers of degenerating axons 2 and 3 weeks after MOG sensitization. **c** and **d**: Wld mice had no or only few damaged axons 2 and 3 weeks after MOG sensitization. **e** and **f**: Axonal swelling was obvious 1 month after MOG sensitization, and high levels of axonal degeneration were seen 2 months after MOG sensitization. Original magnifications, $\times 115$.

also looked for neuropathological changes in Wld mice 5 months after sensitization and found no difference between the 5 months, and 1 and 2 months after sensitization, time points.

In addition, we compared the development of axonal degeneration using immunohistochemistry against nonphosphorylated neurofilament.^{9,12} In B6 mice, swollen axons positive for nonphosphorylated neurofilament were evident at 2 and 3 weeks after sensitization (Figure 3, **a** and **b**). In contrast, axonal degeneration was slow in Wld mice (Figure 3, **c** and **d**). Axonal degeneration was not obvious until 1 month after sensitization, and extensive axonal swelling was observed 2 months after sensitization (Figure 3, **e** and **f**). Interestingly, at this time point, Wld mice had no clinical signs, and demyelinating lesions, if present, were small, despite the fact that numerous damaged axons were present histologically.

MOG-Specific Lymphoproliferative Responses

We next examined whether MOG-specific lymphoproliferative responses were associated with clinical and histological observations. Two weeks after sensitization, both B6 and Wld mice had similarly high T-cell proliferative responses to MOG₃₅₋₅₅ (Figure 4a). After 3 weeks,

Figure 4. Lymphoproliferative responses in EAE mice induced with MOG. **a:** We induced EAE in B6 mice (closed column) and Wld mice (open column) and examined lymphoproliferative responses against MOG 2 and 3 weeks and 1 and 2 months after MOG sensitization. Both mouse strains had comparable high MOG-specific T-cell proliferation 2 weeks after sensitization. After 3 weeks, MOG-specific responses were enhanced in B6 mice, whereas those in Wld mice declined. There was no difference in [^{3} H]thymidine incorporation in wells with no stimulation (spontaneous proliferation) between the mouse strains (mean cpm $\pm$ SEM: B6 mice, 268 ± 26 ; Wld mice, 276 ± 28). **b:** During the chronic stage of EAE in B6 mice, 2 to 3 months after MOG sensitization, MNCs from regional lymph nodes were cultured with MOG or MBP in the presence or absence of CD4, MHC class II, or CD8 antibody. CD4 and MHC class II antibodies completely blocked MOG-specific lymphoproliferation, whereas CD8 antibody had no effect. MBP-specific lymphoproliferation was not detected. Lymph nodes were pooled from two to three mice. Results are average of two pools and representative of two independent experiments.

however, anti-MOG T-cell proliferation declined in Wld mice, whereas T cells from B6 mice had enhanced anti-MOG responses during the chronic stage. Incubation with CD4 and MHC class II antibodies completely inhibited MOG-specific responses, whereas incubation with CD8 antibody had no effect on MOG-specific lymphoproliferation (Figure 4b). We were not able to detect lymphoproliferative responses against MBP.

Figure 5. Clinical signs of TMEV infection. **a:** B6 and Wld mice were infected with TMEV. Only a small percentage of B6 mice (filled circle) developed paralysis during the acute stage. In contrast, 30% and 50% of TMEV-infected Wld mice showed limb paralysis during the acute and chronic stages, respectively. Shown are percentages of mice that had paralysis among 63 B6 mice and 57 Wld mice infected with TMEV in two independent experiments. $**P < 0.01$, compared with B6 mice. During the acute stage, Wld mice (**b**) developed flaccid paralysis of the hind limb, and some Wld mice (**c**) showed impaired movement of the proximal, but not distal, part of the limb, resulting in a gait disturbance that resembled sea lions. Note that the feet were visible from the top in paralyzed mice (**c**) but not in mice without paralysis (**e**). **d:** Some Wld mice developed flaccid tail paralysis.

Clinical Course of TMEV Infection

We infected B6 and Wld mice with TMEV and followed the mice for clinical signs for 6 months. B6 mice are known to

Figure 6. Neuropathology of B6 (**a**, **c**) and Wld (**b**, **d**) mice, 3 weeks after TMEV infection. In the hippocampus, B6 mice had no lesions in the hippocampus (**a**), whereas Wld mice developed substantial perivascular cuffings (**arrows**) (**b**). In consecutive sections, we detected no virus antigen-positive cells in B6 mice (**c**), and virus persistence in Wld mice (**d**). **a** and **b**: Luxol fast blue staining. **c** and **d**: Immunohistochemistry against TMEV. Original magnifications: $\times 120$ (**a**, **b**); $\times 370$ (**c**, **d**).

be resistant to TMEV-induced demyelinating disease. During the acute stage, a small percentage of B6 mice developed mild paralysis for only a few days to approximately 1 week after infection (Figure 5a). In contrast, a significantly higher percentage of Wld mice developed paralysis [percentage of paralyzed mice: B6 mice, 8% (five of 63 mice); Wld mice, 30% (17 of 57 mice); $P < 0.01$, χ^2 test] (Figure 5b). Wld mice showed paralysis for a prolonged period of time, and paralysis was more severe than that seen in B6 mice. A few Wld mice developed an unusual pattern of hind limb paralysis in which thigh and leg movement was severely restricted with preservation of foot movement (Figure 5c). Some Wld mice were incontinent and had flaccid paralysis of the tail, which is rare in any strain of mice infected with the DA

strain of TMEV (Figure 5d). Five weeks after infection, all B6 and Wld mice had completely recovered from the acute disease; from this time point, no B6 mice showed clinical signs during the 6-month observation period. In contrast, half of the Wld mice developed limb paralysis during the chronic stage [percentage of paralyzed mice: B6 mice, 0% (0 of 16 mice); Wld mice, 50% (9 of 18 mice); $P < 0.01$, Fisher's exact probability test] (Figure 5a).

Neuropathology of TMEV Infection

In Wld mice infected with TMEV, 1 week after infection, perivascular cuffing was seen in the cerebral cortex,

corpus callosum, septum, hippocampus, and thalamus. We also found extensive neuronal death of the pyramidal cell layer in the hippocampus. Neuronophagia of anterior horn cells was extensive and seen in all levels of the spinal cords. Meningitis was mild to moderate and was comprised of MNCs. Two weeks after infection, substantial inflammation was seen in the same regions as that seen 1 week after infection, but the extent of inflammation was milder than that of 1 week after infection. Hippocampal lesions became gliotic, and cell loss was evident in the pyramidal cell layers. In the spinal cord, neuronophagia was observed in several segments, and some lesions became gliotic. Meningitis was mild. Three and 5 weeks after infection, we still found substantial perivascular cuffing made up of MNCs with gliosis in the hippocampus (Figure 6b), although the number of perivascular cuffs was smaller than that in the acute stage (Figure 7a). In the spinal cord, mild meningitis comprised of MNC and glial stars was present in the white matter of some mice. However, no obvious neuronophagia of anterior horn cells or demyelination in the white matter was observed. Three and 6 months after infection, no lesions were seen in the brain or spinal cord, except that cells were seen collected in the meninges in a few mice.

B6 mice, during the first 2 weeks after TMEV infection, developed a similar neuropathological picture to that of Wld mice. No difference was seen in the number and distribution of perivascular cuffing (Figure 7a). Hemorrhagic lesions were seen in a few mice at 1 week after infection and severe gliosis was seen mainly in the hippocampus of most mice at 2 weeks after infection. In contrast to Wld mice, 3 and 5 weeks after infection, inflammation subsided in B6 mice (Figure 6a). Spinal cord pathology was similar to that of Wld mice. Three and 6 months after infection, no neuropathology except gliosis in the hippocampus was detected.

Viral Antigen-Positive Cells in the CNS

In both mouse strains, viral antigen-positive cells were mainly found in the cerebral cortex and the pyramidal cell layers of the hippocampus. Morphologically, viral antigen-positive cells were neurons. One week after infection, there was no significant difference in the numbers or distribution of viral antigen-positive cells between the two strains (Figure 7b). However, from 2 weeks after infection, the numbers of viral antigen-positive cells were higher in Wld mice than in B6 mice (Figure 6, c and d, and Figure 7b; $P < 0.05$, *t*-test, 2 and 5 weeks after infection). In the spinal cord, small numbers of viral antigen-positive cells were seen in the anterior horns during the acute stage and in the anterior funiculus and the ventral root exit zone during the chronic stage. Wld mice had more viral antigen-positive cells in the spinal cord than B6 mice (Figure 7c; $P < 0.05$, 5 weeks after infection).

CNS Virus Titer

To clarify whether there was a correlation between virus replication and pathology, we titrated infectious virus in

Figure 7. Neuropathology of B6 (closed column) and Wld (open column) mice infected with TMEV, 1, 2, 3, and 5 weeks after infection. **a:** Inflammation scores are means of the numbers of perivascular cuffs in the brain of five to eight mice. Wld mice had more severe inflammation than B6 mice, 2, 3, and 5 weeks after infection (5 weeks after infection, $*P < 0.05$, *t*-test). Values are the means $\pm$ SEM of pooled results from two independent experiments. **b:** In the brain, we detected larger numbers of viral antigen-positive cells in Wld mice than in B6 mice, except 1 week after infection (2 and 5 weeks after infection, $*P < 0.05$, *t*-test). **c:** In the spinal cord, Wld mice had more viral antigen-positive cells than B6 mice at all time points (5 weeks after infection, $*P < 0.05$, *t*-test). Values are mean viral antigen-positive cells per mouse $\pm$ SEM for 5 to 11 mice.

Figure 8. Viral titers in the CNS of mice infected with TMEV. Using plaque assays, we titrated infectious virus from the brains (left) and the spinal cords (right) of B6 (closed column) and Wld (open column) mice, 1, 2, and 3 weeks after infection. Values are mean virus titers $\pm$ SEM for three mice.

the brains and spinal cords of three mice infected with TMEV at weeks 1, 2, 3, and 5 and months 3 and 6 after infection (Figure 8). We were able to detect virus from all CNS tissues harvested from mice at 1, 2, and 3 weeks after infection. In the brain, we found higher amounts of virus from Wld mice 2 and 3 weeks after infection. These results were consistent with the immunohistochemistry data described above. In the spinal cord, there were no significant differences between B6 and Wld mice. During the chronic stage at week 5 and months 3 and 6 after infection, we were not able to isolate virus from most of the 36 CNS tissues tested. Virus was detected from only one brain of B6 mice (5 weeks after infection), three spinal cords of B6 mice (two at 5 weeks and one at 3 months after infection), and two spinal cords of Wld mice (5 weeks after infection).

TMEV-Specific Lymphoproliferative Responses

To determine whether virus-specific immune responses correlated with CNS disease, we examined lymphoproliferative responses of MNCs isolated from spleens of mice at 1, 2, 3, and 5 weeks after infection (Figure 9). Wld mice had higher lymphoproliferative responses against TMEV-APCs than B6 mice at 1 and 2 weeks after infection. From this time point, both mouse strains had low levels of virus-specific lymphoproliferation. Lymphoproliferative responses against live TMEV had similar kinetics to those against TMEV-APCs (data not shown). Virus-specific lymphoproliferation stayed at low levels 2 to 5 months after virus infection in both B6 and Wld mice (data not shown).

Muscle Pathology

Damage in motor neurons can cause muscular pathology, including muscle fiber atrophy by denervation. We examined skeletal muscle of the lower extremities of Wld mice infected with TMEV. Muscle pathology was seen in

Figure 9. Virus-specific lymphoproliferative responses. Spleen MNCs were isolated from B6 (closed column) or Wld (open column) mice at 1, 2, 3, and 5 weeks after TMEV infection. MNCs were stimulated with TMEV-APCs. Wld mice had higher lymphoproliferative responses against TMEV-APCs than B6 mice, 1 and 2 weeks after infection. There was no difference in [³H]thymidine incorporation in wells with no stimulation (spontaneous proliferation) between the mouse strains (mean cpm $\pm$ SEM: B6 mice, 307 $\pm$ 52; Wld mice, 380 $\pm$ 75). All cultures were performed in triplicate. Two to three spleens were pooled, and values are the means of stimulation indexes of two to three pools. Results are representative of two independent experiments.

25 and 63% of Wld mice at 3 and 6 months after TMEV infection, respectively, but not in the early time points of TMEV infection. Cross-sections displayed groups of atrophic muscle fibers of varying sizes, suggesting denervation atrophy (Figure 10, a and b). Many atrophic fibers contained a nucleus not only at its periphery but also in the substance of the muscle fiber (internal nuclei). Polymorphonuclear cell infiltration was seen in the restricted areas of some muscle fibers, although most lesions did not contain a prominent polymorphonuclear cell infiltration. Immunohistochemistry using antibody against TMEV demonstrated no virus antigen-positive cells in the muscle (Figure 10c). In contrast, we did not observe muscle pathology in either age-matched uninfected mice or B6 mice infected with TMEV during acute and chronic stages (Figure 10d). Because inflammation around the ventral root exit zone of the spinal cord could induce neurogenic muscle pathology, we examined muscles from Wld mice with MOG-induced EAE. Despite meningitis and inflammation of the spinal cord, no muscle pathology in Wld mice with EAE was present (data not shown).

Discussion

Although axonal damage has been demonstrated in MS, its precise mechanism is unknown. Previously, we and others proposed two hypotheses for how axons are damaged in demyelinating lesions.^{11,36} The first one is that axons are damaged secondarily to myelin and/or oligodendrocyte damage. In this model, lesions develop from

Figure 10. Transverse sections of skeletal muscles from the lower extremities of Wld mice (**a–c**) and B6 mice (**d**), 6 months after TMEV infection. **a:** Wld mice had groups of atrophic muscle fibers (**arrowhead**). Some muscle fibers of normal size have internal nuclei (**arrow**). **b:** Higher magnification demonstrated rounded atrophic fibers, some of which were so atrophic that they resembled macrophages. **c:** A consecutive section immunostained with antibody against TMEV detected no virus antigen in the muscle. **d:** Muscles from B6 mice infected with TMEV had no pathology. All nuclei of the muscle fibers were seen at the periphery of the fibers. **a, b,** and **d:** H&E staining. **c:** Immunohistochemistry against TMEV. Original magnifications: $\times 120$ (**a, c, d**); $\times 360$ (**b**).

the outside (myelin) to the inside (axon) (Outside-In model). On the other hand, in spinal cord injury³⁷ and TMEV infection, axonal damage precedes demyelination, in which lesions develop from the inside (axon) to the outside (myelin) (Inside-Out model). Similar pathology has been described in other viral infections in the CNS, including murine hepatitis virus infection.³⁸

In our experiments, both B6 and Wld mice with EAE had a similar disease onset and severity during the first 2 weeks after MOG sensitization. MOG-specific T-cell proliferative responses were also similar between the two mouse strains during this period. We expected these results, because no immunological deficit has been reported in Wld mice. However, from 3 weeks after MOG sensitization, only B6 mice had enhanced anti-MOG immune responses with severe axonal damage and demyelination. This suggests associations among axonal degeneration, enhanced anti-MOG immune responses and disease progression in B6 mice. Although MOG-specific CD8⁺ T-cell responses have been reported in B6 mice,³⁹ we could not detect CD8⁺ T cells specific for MOG in B6 mice. We also tested whether epitope spreading oc-

curred in B6 mice, because B6 can mount T-cell responses against MBP.^{40,41} However, we did not detect lymphoproliferative responses against MBP in B6 mice. Thus, enhancement of MOG-specific responses in B6 mice was attributable to enhancement of MHC class II restricted CD4⁺ T-cell proliferation, and neither MOG-specific CD8⁺ T-cell proliferation nor MBP-specific T-cell proliferation was detected.

Although we do not know the precise mechanism, we hypothesize the following scenario in EAE progression (Figure 11a). After induction of EAE, anti-MOG autoimmune responses initially attack myelin, and axons are injured by the extreme inflammation secondarily (Outside-In model). Because axonal injury is severe, the distal stumps of axons degenerate (Wallerian degeneration). This results in microglia activation and oligodendrocyte apoptosis along the degenerated tract, leading to secondary demyelination in areas, distant from the original lesion.^{32,42,43} Here, pathology develops from the inside to the outside (Inside-Out model). Degenerated myelin and oligodendrocytes would then be phagocytosed by activated macrophages and microglia, leading to antigen

Figure 11. Possible mechanisms of disease progression in B6 and Wld mice with EAE (**a**) and TMEV infection (**b**). **a:** In B6 mice with EAE, anti-myelin autoimmune T cells first damage myelin from the outside, leading to secondary axonal damage (orange), in which lesions develop from the outside to the inside (Outside-In model). If axonal damage is severe, this will lead to Wallerian degeneration of the distal stumps of axons. Then, myelin sheaths that wrap degenerated axons would degenerate secondarily, and be phagocytized by microglia/macrophages. Here, lesions develop from the inside (axon) to the outside (myelin) (Inside-Out model). Myelin antigen will be presented by APCs and further activate myelin-specific T cells, leading to the second cascade reaction and disease development. In Wld mice, although MOG sensitization induces a similar level of anti-myelin autoimmune responses and neuropathology, a lack of or slow Wallerian degeneration intervenes in the vicious cycle (red open arrow), which results in suppression of disease progression. **b:** TMEV (blue) spreads in the CNS via axonal transport. In B6 mice, induction of axonal degeneration prevents virus dissemination in the CNS. In Wld mice, axonal preservation favors virus dissemination and persistence in the CNS.

presentation in the CNS. This leads to enhanced (induced) myelin-specific autoimmune responses, which can attack myelin from the outside (Outside-In model), and trigger a second cascade of inflammation. Therefore, the Inside-Out and Outside-In models act in synergy and are not mutually exclusive.

In Wld mice, although MOG immunization induced a similar degree of anti-myelin autoimmune responsiveness and neuropathology 2 weeks after sensitization, a lack of or a delay in Wallerian degeneration could prevent the inflammatory cascade (Figure 11a, open red arrow), resulting in suppression of the enhancement of anti-myelin immune responses and disease progression. During the chronic stage, Wld mice developed axonal degener-

ation, but this did not lead to an exacerbation of demyelinating disease 2 months after EAE induction. This is likely attributable to anti-MOG T-cell responses that have subsided in Wld mice at this chronic stage. Here, even though axonal degeneration begins to occur at 2 months, there is no (activated) MOG-specific T cells present in the periphery to be recruited into the CNS. Another possibility is that regulatory T (Treg) cells are present at this later time point that would modulate the MOG-specific memory T cells. Thus, no enhancement of anti-MOG-specific responses occurs, although myelin antigen might be presented to axonal degeneration. If this hypothesis is true, treatment that intervenes at any of the steps of the cascade reaction and causes a long enough delay in advancement to the following steps would prevent disease progression. We are currently testing this and the presence of MOG-specific Treg cells at 2 months after sensitization.

In most EAE models and MS patients, disease courses are either monophasic or relapsing-remitting, and only a small percentage of MS patients have continuous disease progression. The lack of disease progression might be attributable to generation of Treg cells, Th2 cells, apoptotic elimination of myelin-specific T cells, and preservation of axons, all of which potentially stop or delay the inflammatory cascade described in Figure 11a.

Theoretically, the Outside-In and Inside-Out models can form a vicious cycle, independent of whichever event starts first. Thus, we wondered if a lack of Wallerian degeneration could also be beneficial in TMEV infection, in which lesions develop from the inside (axon) to the outside (myelin) (Inside-Out model). We found that, during TMEV infection, neuropathology and virus replication in the CNS were similar between B6 and Wld mice 1 week after TMEV infection. However, 3 weeks after infection, clinical signs, substantial inflammation, and virus persistence were present only in Wld mice. Because TMEV is known to infect neurons and to spread using axonal flow during the acute stage of infection, delayed axonal degeneration in Wld mice could favor virus transport and persistence in the CNS (Figure 11b). Active virus spread and replication in Wld mice most likely resulted in enhanced TMEV-specific lymphoproliferative responses compared with those measured in B6 mice. In contrast, rapid induction of axonal degeneration in B6 mice would prevent virus dissemination and persistence in the CNS (Figure 11b). This hypothesis is in accord with our previous finding that the DA strain of TMEV induced obvious axonal degeneration 2 weeks after infection.¹² Thus, in B6 mice, virus may spread in axons during the first week of TMEV infection, but induction of axonal degeneration at 2 to 3 weeks after infection could prevent further virus spread in the CNS. If this hypothesis is correct, Wallerian degeneration might be a self-destructive defense mechanism that protects from the transport of toxic substances, including virus, in the CNS.¹⁹

TMEV induced a biphasic disease in Wld mice. The paralytic disease seen during the first 5 weeks of infection can be explained by prolonged gray matter inflammation (polioencephalomyelitis) with active virus spread

and persistence in neurons and axons. However, during the late chronic stage of TMEV infection, we found muscle pathology but no CNS pathology, such as demyelination, or virus persistence in mice with paralysis. Thus, the late chronic disease in Wld mice is markedly different from demyelinating disease in susceptible mice infected with TMEV.

Rustigian and Pappenheimer⁴⁴ first described muscle pathology in TMEV-infected Swiss mice. Intramuscular injection of TMEV caused acute myositis with virus replication that peaked at 6 days after infection. GDVII and FA viruses of the GDVII subgroup caused severe myositis, whereas 4727 virus and FV virus of the TO subgroup produced mild and no myositis, respectively. Daniels and colleagues⁴⁵ demonstrated that the DA strain of TMEV also caused acute myositis after intramuscular injection, but intracerebral injection of DA virus caused myositis only in suckling mice but not in mice inoculated at the age of 3 weeks or greater. Intraperitoneal infection of the DA strain of TMEV has been reported to induce cardiac and skeletal muscle disease in various strains of 10- to 14-day-old mice, including B6 mice.⁴⁶ In this model, severe necrotizing myositis was comprised of T cells, peaking at 3 weeks after infection, having characteristics of muscle pathology. In the muscle, virus antigen was detectable during the acute stage of TMEV infection. In summary, muscle pathology in TMEV infection reported previously is characterized by acute inflammation with virus replication in muscle. In contrast, Wld mice developed muscle pathology during the late chronic stage with no virus persistence. Thus, the type of muscle disease in Wld mice is different and novel from what has been previously reported in TMEV infection.

The late paralytic disease in Wld mice infected with TMEV has similarities to postpolio syndrome (PPS). Dalkas⁴⁷ defined PPS as the development of new muscle weakness and fatigue in skeletal or bulbar muscles, unrelated to any known cause, that begins 25 to 30 years after an acute attack of paralytic poliomyelitis. Poliomyelitis is caused by poliovirus, which belongs to the same family as TMEV, *Picornaviridae*. Risk factors for the development of PPS include the severity of the acute poliomyelitis.⁴⁸ The cause of PPS is unknown. The leading hypothesis is that an excessive metabolic stress on remaining motor neurons throughout many years eventually results in the dropout of the new nerve terminals and the motor neurons themselves. The role of virological or immunological mechanisms in the development of PPS has not yet been substantiated. In the muscle, a mixture of myopathic and neuropathic features including group atrophy and nuclear clumps has been reported.^{49,50} Perivascular or interstitial inflammatory cells were also found in some patients. Thus, both clinically and pathologically, PPS is very similar to the late paralytic disease seen in Wld mice infected with TMEV.

B6 mice are known to be resistant to TMEV-induced demyelinating disease. Although a lack of axonal degeneration seemed to be beneficial in TMEV infection in B6 mice, this might not be the case in which TMEV infection occurs in susceptible strains, such as SJL/J mice. TMEV-infected SJL/J mice can develop not only anti-virus im-

mune responses but also anti-myelin immune responses such as antibody against galactocerebroside and T-cell proliferative responses against myelin proteolipid protein (PLP), by molecular mimicry and epitope spreading.⁵¹ These anti-myelin immune responses have been suggested to exacerbate demyelination induced by TMEV. Thus, in TMEV-susceptible mice, viral persistence as well as immune responses against virus and myelin antigens can play an important role in demyelination. In this scenario, preservation of axons can be a double-edged sword. Preservation of axons could interrupt a cascade reaction composed of anti-myelin immune responses and axonal degeneration, but at the same time, it could favor virus spread in the CNS. Because TMEV disseminates within axons only during the acute stage, and epitope spreading to the myelin antigens occurs only during the late chronic stage, preservation of axons could be detrimental during the acute stage, but be beneficial during the late chronic stage in susceptible mice infected with TMEV. This can be addressed in future experiments, such as inhibition of axonal transport only during the acute stage, or using mice with the Wld mutation in a TMEV-susceptible genetic background.

It is controversial which parts of the *Wld^S* gene are required for preservation of axons in Wld mice.⁵² For example, *Nmnat 1* has been reported to be sufficient for axon protection *in vitro*, whereas transgenic mice that express *Nmnat 1* show a normal rate of Wallerian degeneration.⁵² *Wld^S* protein has also been suggested to exert its protective effects by modulating expression levels of genes and proteins that normally execute a program of axon degeneration.⁵³ Wang and colleagues⁵⁴ reported that delivery of the *Wld^S* gene product could transfer a neuroprotective phenotype *in vitro*, suggesting that *Wld^S* gene therapy might provide a beneficial effect for patients with axonal degeneration. Therefore, experiments using Wld mice should not only help to elucidate roles of axonal injury, but clarify the role of the *Wld^S* gene product and its therapeutic potential in axonal degeneration. However, in our current study, we demonstrated that axonal degeneration could play contrasting roles (beneficial versus detrimental) depending on the initiator of disease, autoimmune versus virus. Thus, caution should be taken with a therapeutic strategy targeting axonal degeneration in the future.

Acknowledgments

We thank Jane E. Libbey, M.S., for many helpful discussions; Sarah E. Doyle, B.S., Faris Hasanovic, Daniel Scott Kennett, Nikki Jo Kirkman, B.S., Benjamin J. Marble, J. Wes Peterson, Daniel G. Smith, Steven R. Wheelwright, and Nathan J. Young, B.S., for excellent technical assistance; and Ms. Kathleen Borick for the outstanding preparation of the manuscript.

References

1. Antony JM, van Marle G, Opii W, Butterfield DA, Mallet F, Yong VW, Wallace JL, Deacon RM, Warren K, Power C: Human endogenous

- retrovirus glycoprotein-mediated induction of redox reactants causes oligodendrocyte death and demyelination. *Nat Neurosci* 2004, 7:1088–1095
2. Kennedy PGE: Neurological aspects of lentiviral infections in animals. Neurological aspects of human retroviruses. Bailliere's Clinical Neurology. Edited by P Rodge. Amsterdam, Elsevier, 1992, pp 41–59
3. Kennedy PG, Steiner I: On the possible viral aetiology of multiple sclerosis. *QJM* 1994, 87:523–528
4. Opsahl ML, Kennedy PGE: Early and late HHV-6 gene transcripts in multiple sclerosis lesions and normal appearing white matter. *Brain* 2005, 128:516–527
5. Tsunoda I, Fujinami RS: Two models for multiple sclerosis: experimental allergic encephalomyelitis and Theiler's murine encephalomyelitis virus. *J Neuropathol Exp Neurol* 1996, 55:673–686
6. Tsunoda I, Fujinami RS: Theiler's murine encephalomyelitis virus. Persistent Viral Infections. Edited by R Ahmed, I Chen. Chichester, John Wiley & Sons Ltd., 1999, pp 517–536
7. Lassmann H: Multiple sclerosis pathology: evolution of pathogenetic concepts. *Brain Pathol* 2005, 15:217–222
8. Silber E, Sharief MK: Axonal degeneration in the pathogenesis of multiple sclerosis. *J Neurol Sci* 1999, 170:11–18
9. Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mörk S, Bö L: Axonal transection in the lesions of multiple sclerosis. *N Engl J Med* 1998, 338:278–285
10. Ferguson B, Matyszak MK, Esiri MM, Perry VH: Axonal damage in acute multiple sclerosis lesions. *Brain* 1997, 120:393–399
11. Tsunoda I, Fujinami RS: Inside-Out versus Outside-In models for virus induced demyelination: axonal damage trigger demyelination. *Springer Semin Immunopathol* 2002, 24:105–125
12. Tsunoda I, Kuang L-Q, Libbey JE, Fujinami RS: Axonal injury heralds virus-induced demyelination. *Am J Pathol* 2003, 162:1259–1269
13. Racke MK, Hawker K, Frohman EM: Multiple sclerosis and magnetic resonance imaging: advancement in linking the pictures with the progression. *Arch Neurol* 2001, 58:35–36
14. Adams RD, Kubik CS: The morbid anatomy of the demyelinating diseases. *Am J Med* 1952, 12:510–546
15. Evangelou N, Konz D, Esiri MM, Smith S, Palace J, Matthews PM: Regional axonal loss in the corpus callosum correlates with cerebral white matter lesion volume and distribution in multiple sclerosis. *Brain* 2000, 123:1845–1849
16. Simon JH, Kinkel RP, Jacobs L, Bub L, Simonian N: A Wallerian degeneration pattern in patients at risk for MS. *Neurology* 2000, 54:1155–1160
17. Tortorella P, Rocca MA, Mezzapesa DM, Ghezzi A, Lamantia L, Comi G, Filippi M: MRI quantification of gray and white matter damage in patients with early-onset multiple sclerosis. *J Neurol* 2006, 253:903–907
18. Coleman MP, Perry VH: Axon pathology in neurological disease: a neglected therapeutic target. *Trends Neurosci* 2002, 25:532–537
19. Gillingwater TH, Ribchester RR: Compartmental neurodegeneration and synaptic plasticity in the Wild^s mutant mouse. *J Physiol* 2001, 534:627–639
20. Perry VH, Lunn ER, Brown MC, Cahusac S, Gordon S: Evidence that the rate of Wallerian degeneration is controlled by a single autosomal dominant gene. *Eur J Neurosci* 1990, 2:408–413
21. Glass JD, Griffin JW: Neurofilament redistribution in transected nerves: evidence for bidirectional transport of neurofilaments. *J Neurosci* 1991, 11:3146–3154
22. Glass JD, Griffin JW: Retrograde transport of radiolabeled cytoskeletal proteins in transected nerves. *J Neurosci* 1994, 14:3915–3921
23. Watson DF, Glass JD, Griffin JW: Redistribution of cytoskeletal proteins in mammalian axons disconnected from their cell bodies. *J Neurosci* 1993, 13:4354–4360
24. Mack TGA, Reiner M, Beirowski B, Mi W, Emanuelli M, Wagner D, Thomson D, Gillingwater T, Court F, Conforti L, Fernando FS, Tarlton A, Andressen C, Addicks K, Magni G, Ribchester RR, Perry VH, Coleman MP: Wallerian degeneration of injured axons and synapses is delayed by a Ube4b/Nmnat chimeric gene. *Nat Neurosci* 2001, 4:1199–1206
25. Araki T, Sasaki Y, Milbrandt J: Increased nuclear NAD biosynthesis and SIRT1 activation prevent axonal degeneration. *Science* 2004, 305:1010–1013
26. Wang J, Zhai Q, Chen Y, Lin E, Gu W, McBurney MW, He Z: A local mechanism mediates NAD-dependent protection of axon degeneration. *J Cell Biol* 2005, 170:349–355
27. Mendel I, Kerlero de Rosbo N, Ben-Nun A: A myelin oligodendrocyte glycoprotein peptide induces typical chronic experimental autoimmune encephalomyelitis in H-2^b mice: fine specificity and T cell receptor Vb expression of encephalitogenic T cells. *Eur J Immunol* 1995, 25:1951–1959
28. Tsunoda I, Wada Y, Libbey JE, Cannon TS, Whitby FG, Fujinami RS: Prolonged gray matter disease without demyelination caused by Theiler's murine encephalomyelitis virus with a mutation in VP2 puff B. *J Virol* 2001, 75:7494–7505
29. Zurbriggen A, Fujinami RS: Theiler's virus infection in nude mice: viral RNA in vascular endothelial cells. *J Virol* 1988, 62:3589–3596
30. Tsunoda I, McCright IJ, Kuang L-Q, Zurbriggen A, Fujinami RS: Hydrocephalus in mice infected with a Theiler's murine encephalomyelitis virus variant. *J Neuropathol Exp Neurol* 1997, 56:1302–1313
31. Deibler GE, Martenson RE, Kies MW: Large scale preparation of myelin basic protein from central nervous tissue of several mammalian species. *Prep Biochem* 1972, 2:139–165
32. Tsunoda I, Kuang L-Q, Fujinami RS: Induction of autoreactive CD8⁺ cytotoxic T cells during Theiler's murine encephalomyelitis virus infection: implications for autoimmunity. *J Virol* 2002, 76:12834–12844
33. Janeway Jr CA, Conrad PJ, Lerner EA, Babich J, Wettstein P, Murphy DB: Monoclonal antibodies specific for Ia glycoproteins raised by immunization with activated T cells: possible role of T cellbound Ia antigens as targets of immunoregulatory T cells. *J Immunol* 1984, 132:662–667
34. Tsunoda I, Kuang L-Q, Tolley ND, Whitton JL, Fujinami RS: Enhancement of experimental allergic encephalomyelitis (EAE) by DNA immunization with myelin proteolipid protein (PLP) plasmid DNA. *J Neuropathol Exp Neurol* 1998, 57:758–767
35. Tsunoda I, Kuang L-Q, Iggen IZM, Fujinami RS: Converting relapsing remitting to secondary progressive experimental allergic encephalomyelitis (EAE) by ultraviolet B irradiation. *J Neuroimmunol* 2005, 160:122–134
36. Maggs FG, Palace J: The pathogenesis of multiple sclerosis: is it really a primary inflammatory process? *Mult Scler* 2004, 10:326–329
37. Abe Y, Yamamoto T, Sugiyama Y, Watanabe T, Saito N, Kayama H, Kumagai T: Apoptotic cells associated with Wallerian degeneration after experimental spinal cord injury: a possible mechanism of oligodendroglial death. *J Neurotrauma* 1999, 16:945–952
38. Dandekar AA, Wu GF, Pewe L, Perlman S: Axonal damage is T cell mediated and occurs concomitantly with demyelination in mice infected with a neurotropic coronavirus. *J Virol* 2001, 75:6115–6120
39. Sun D, Whitaker JN, Huang Z, Liu D, Coleclough C, Wekerle H, Raine CS: Myelin antigen-specific CD8⁺ T cells are encephalitogenic and produce severe disease in C57BL/6 mice. *J Immunol* 2001, 166:7579–7587
40. Furlan R: MBP-specific experimental autoimmune encephalomyelitis in C57BL/6 mice. *J Immunol* 2004, 173:5–6
41. Shaw MK, Kim C, Hao HW, Chen F, Tse HY: Induction of myelin basic protein-specific experimental autoimmune encephalomyelitis in C57BL/6 mice: mapping of T cell epitopes and T cell receptor VB gene segment usage. *J Neurosci Res* 1996, 45:690–699
42. Konno H, Yamamoto T, Iwasaki Y, Suzuki H, Saito T, Terunuma H: Wallerian degeneration induces Ia-antigen expression in the rat brain. *J Neuroimmunol* 1989, 25:151–159
43. Konno H, Yamamoto T, Suzuki H, Yamamoto H, Iwasaki Y, Ohara Y, Terunuma H, Harata N: Targeting of adoptively transferred experimental allergic encephalitis lesion at the sites of Wallerian degeneration. *Acta Neuropathol (Berl)* 1990, 80:521–526
44. Rustigian R, Pappenheimer AM: Myositis in mice following intramuscular injection of viruses of the mouse encephalomyelitis group and of certain other neurotropic viruses. *J Exp Med* 1949, 89:69–92
45. Daniels JB, Pappenheimer AM, Richardson S: Observations on encephalomyelitis of mice (DA strain). *J Exp Med* 1952, 96:517–535
46. Gómez RM, Rinehart JE, Wollmann R, Roos RP: Theiler's murine encephalomyelitis virus-induced cardiac and skeletal muscle disease. *J Virol* 1996, 70:8926–8933
47. Dalakas MC: The post-polio syndrome as an evolved clinical entity. Definition and clinical description. *Ann NY Acad Sci* 1995, 753:68–80
48. Jubelt B, Agre JC: Characteristics and management of postpolio syndrome. *JAMA* 2000, 284:412–414

49. Dalakas MC: Morphologic changes in the muscles of patients with postpoliomyelitis neuromuscular symptoms. *Neurology* 1988, 38:99–104
50. Borg K, Eström L: Muscle fiber morphology in post-polio patients. *Post-Polio Syndrome*. Edited by LS Halstead, G Grimby. Philadelphia, Hanley & Belfus, Inc., 1995, pp 25–33
51. Tsunoda I, Fujinami RS: TMEV and neuroantigens: myelin genes and proteins, molecular mimicry, epitope spreading, and autoantibody-mediated remyelination. *Experimental Models of Multiple Sclerosis*. Edited by E Lavi, CS Constantinescu. New York, Springer, 2005, pp 593–616
52. Coleman M: Axon degeneration mechanisms: commonality amid diversity. *Nat Rev Neurosci* 2005, 6:889–898
53. Gillingwater TH, Wishart TM, Chen PE, Haley JE, Robertson K, MacDonald SH-F, Middleton S, Wawrowski K, Shipston MJ, Melmed S, Wyllie DJA, Skehel PA, Coleman MP, Ribchester RR: The neuroprotective *Wld^S* gene regulates expression of PTTG1 and erythroid differentiation regulator 1-like gene in mice and human cells. *Hum Mol Genet* 2006, 15:625–635
54. Wang MS, Fang G, Culver DG, Davis AA, Rich MM, Glass JD: The *Wld^S* protein protects against axonal degeneration: a model of gene therapy for peripheral neuropathy. *Ann Neurol* 2001, 50:773–779