Differential T cell response in central and peripheral nerve injury: connection with immune privilege

GILA MOALEM,*,† ALON MONSONEGO,* YAEL SHANI,* IRUN R. COHEN,† AND MICHAL SCHWARTZ*,[1]

*Department of Neurobiology, The Weizmann Institute of Science, 76100 Rehovot, Israel; and †Department of Immunology, The Weizmann Institute of Science, 76100 Rehovot, Israel

ABSTRACT **The central nervous system (CNS), unlike the peripheral nervous system (PNS), is an immune-privileged site in which local immune responses are restricted. Whereas immune privilege in the intact CNS has been studied intensively, little is known about its effects after trauma. In this study, we examined the influence of CNS immune privilege on T cell response to central nerve injury. Immunocytochemistry revealed a significantly greater accumulation of endogenous T cells in the injured rat sciatic nerve than in the injured rat optic nerve (representing PNS and CNS white matter trauma, respectively). Use of the *in situ* terminal deoxytransferase-catalyzed DNA nick end labeling (TUNEL) procedure revealed extensive death of accumulating T cells in injured CNS nerves as well as in CNS nerves of rats with acute experimental autoimmune encephalomyelitis, but not in injured PNS nerves. Although Fas ligand (FasL) protein was expressed in white matter tissue of both systems, it was more pronounced in the CNS. Expression of major histocompatibility complex (MHC) class II antigens was found to be constitutive in the PNS, but in the CNS was induced only after injury. Our findings suggest that the T cell response to central nerve injury is restricted by the reduced expression of MHC class II antigens, the pronounced FasL expression, and the elimination of infiltrating lymphocytes through cell death.—Moalem, G., Monsonego, A., Shani, Y., Cohen, I. R., Schwartz, M. Differential T cell response in central and peripheral nerve injury: connection with immune privilege. *FASEB J.* 13, 1207–1217 (1999)**

Key Words: T lymphocytes · CNS · PNS · Fas ligand · major histocompatibility complex

MAINTENANCE OF NEURAL functions is vital for vertebrate survival, and loss of these functions as a result of local immune responses may threaten central nervous system (CNS)[2] integrity. To maintain proper neural functioning, the CNS may have had to evolve mechanisms that limit its vulnerability to irreversible modifications that might be caused by immune reactions. The CNS, in contrast to the peripheral nervous system (PNS), is an immune-privileged site (1, 2). The concept of CNS immune privilege is supported by the prolonged survival of allografts within the brain parenchyma (3, 4), the presence of a blood-brain barrier (5), the absence of typical lymphatic drainage (6), the reduced expression of major histocompatibility complex (MHC) class I and II antigens (7, 8), unconventional antigen-presenting cells (9), a low concentration of complement components (10), and immunosuppression by the CNS microenvironment (11, 12). Immune privilege in the CNS was long thought to be maintained by 'immune ignorance' (13). It was believed that the CNS is isolated from the immune system, preventing antigen escape and excluding immune cells, and that consequently the immune system simply ignores the area. Over the years it has become clear that antigens from the CNS can escape and induce immune responses in the periphery (10, 14), and that the CNS is accessible to activated antigen-specific lymphocytes (15). Since antigens originating in the CNS can reach and influence the systemic immune apparatus and because immune cells can gain access to the CNS, immune ignorance is no longer a valid explanation of CNS privilege.

Studies of CNS inflammation caused by autoimmune myelin-specific T cells in different states of activation revealed that the blood-brain barrier effectively prevents resting T cells from entering the CNS parenchyma. These studies demonstrated, however, that activated myelin-specific T cells are able to pass

[1] Correspondence: E-mail: bnschwar@weizmann.weizmann.ac.il

[2] Abbreviations: BSA, bovine serum albumin; CNP, cyclic nucleotide phosphohydrolase; CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; FasL, Fas ligand; FCS, fetal calf serum; GFAP, glial fibrillary acidic protein; IFN, interferon; IgG, immunoglobulin G; IL, interleukin; i.p., intraperitoneal; MBP, myelin basic protein; MHC, major histocompatibility complex; PBS, phosphate-buffered saline; PDL, poly-D-lysine; PMSF, phenylmethylsulfonylfluoride; PNS, peripheral nervous system; RPL19, ribosomal protein L19; RT-PCR, reverse transcriptase-polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TCR, T cell receptor; TUNEL, terminal deoxytransferase-catalyzed DNA nick end labeling.

0892-6638/99/0013-1207/$02.25 © FASEB

through the blood-brain barrier and initiate CNS lesions causing autoimmune disease (16), thus suggesting that access to the CNS is restricted to T cells that are activated (9). It was further shown that activated T cells, irrespective of their antigen specificity, enter the CNS parenchyma within hours of injection, but only cells capable of reacting with a CNS antigen can persist there (15).

After CNS injury, the recruitment of macrophages is delayed and limited compared with the strong macrophage response after PNS injury (17). CNS injury may also be accompanied by the infiltration of T lymphocytes into the site of the lesion (18). Although such infiltration might imply the possibility of a classic immune response within the damaged CNS, the effect of the immune-privileged status on these infiltrating T cells is not yet known. In the present study, we examined whether the dialog between T cells and the CNS differs from the T cell–PNS dialog in response to injury. Using partial crush injuries of the optic and sciatic nerves as models for CNS and PNS white matter trauma, respectively, we demonstrate differential T cell response to injury of the central and peripheral nerves. In the injured sciatic nerve, T cell accumulation is significantly greater than in the injured optic nerve. Elimination of T cells through cell death occurs extensively in the optic nerve after injury and in rats with experimental autoimmune encephalomyelitis (EAE), but only to a very small extent in the injured sciatic nerve. Moreover, MHC class II antigens are constitutively expressed in the sciatic nerve, but are induced only after injury in the optic nerve. Fas ligand (FasL) mRNA is expressed in both optic and sciatic nerves, but FasL protein is more pronounced in the optic nerve. These results suggest that the immune-privileged CNS restricts the T cell response to nerve injury.

MATERIALS AND METHODS

Animals

Inbred female Lewis rats (8–12 wk old) and newborn Wistar or Lewis rats were supplied by the Animal Breeding Center of the Weizmann Institute of Science. The rats were housed in a light- and temperature-controlled room and matched for age and sex in each experiment. Animals were used according to the regulations formulated by the Institutional Animal Care and Use Committee.

T cells

A T cell line specific for myelin basic protein (MBP) was generated from draining lymph node cells obtained from Lewis rats immunized with MBP antigen, which was prepared from guinea pig spinal cord as described previously (19). MBP was dissolved in 1 mg/ml of phosphate-buffered saline (PBS) and emulsified with an equal volume of Freund's incomplete adjuvant (Difco Laboratories, Detroit, Mich.) supplemented with 4 mg/ml of *Mycobacterium tuberculosis* (Difco). Ten days after the antigen was injected into their hind foot pads in 0.1 ml of the emulsion, the rats were killed and their draining lymph nodes were surgically removed and dissociated. The cells were washed and activated with MBP antigen (10 μg/ml) in proliferation medium containing Dulbecco's modified Eagle's medium (DMEM) supplemented with L-glutamine (2 mM), 2-mercaptoethanol (5×10^{-5} M), sodium pyruvate (1 mM), penicillin (100 IU/ml), streptomycin (100 μg/ml), nonessential amino acids (1 ml/100 ml), and autologous rat serum 1% (v/v). After incubation for 72 h at 37°C, 90% relative humidity, and 7% CO_2, the cells were transferred to propagation medium consisting of DMEM, L-glutamine, 2-mercaptoethanol, sodium pyruvate, nonessential amino acids, and antibiotics in the same concentrations as above, with the addition of 10% fetal calf serum (FCS) (v/v) and 10% T cell growth factor derived from the supernatant of concanavalin A-stimulated spleen cells. Cells were grown in propagation medium for 4–10 days before being restimulated with their antigen (10 μg/ml) in the presence of irradiated (2000 rad) thymus cells (10^7 cells/ml) in proliferation medium. The T cell line was expanded by repeated stimulation and propagation (20).

Glial cells

Primary cultures of glial cells were prepared by a modification of the procedure of McCarthy and de Vellis (21). Cells dissociated from the cerebral cortex of 2-day-old rats were cultured in poly-D-lysine (PDL) -coated tissue culture flasks (2 brains/85 cm^2 flask) containing DMEM, 2 mM glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10% FCS. The medium was changed after 24 h and every 2 days thereafter. To obtain pure cultures of microglia, after 8 days the flasks were shaken at 37°C on a rotary platform for 6 h and the detached cells were collected and seeded on PDL-coated coverslips in 24-well plates (10^5 cells/ml in each well) in RPMI 1640 medium supplemented with 2 mM glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, 5×10^{-5} M 2-mercaptoethanol, and 10% FCS. Fresh medium was added to the flasks, which were then shaken for an additional 18 h at 37°C. The detached cells, consisting mostly of progenitor cells for oligodendrocytes and type 2 astrocytes, were collected and seeded on PDL-coated coverslips in 24-well plates (5×10^4 cells/ml in each well). To encourage oligodendrocyte development, seeding was carried out in Raff's modification of Bottenstein and Sato's defined medium (22, 23). Fresh medium was again added to the flasks, and 50 μl of 25 mM cytosine-β-D-arabinofuranoside (Sigma, St. Louis, Mo.) was added 1 day later. After 24 h, the medium was replaced by a defined medium for astrocytes consisting of DMEM, 2 mM glutamine, 0.1 mg/ml transferrin, 0.1% free fatty acid bovine serum albumin (BSA), 0.1 mM putrescine, 0.45 mM L-thyroxine, and 0.224 mM sodium selenite. The astrocytes were trypsinized and plated on PDL-coated coverslips in 24-well plates (5×10^4 cells/ml in each well).

Crush injury of optic and sciatic nerves

Crush injury of the optic nerve was performed as described previously (24, 25). Briefly, rats were deeply anesthetized by intraperitoneal (i.p.) injection of Rompun (xylazine, 10 mg/kg; Vitamed, Bat-Yam, Israel) and Vetalar (ketamine, 50 mg/kg; Fort Dodge Laboratories, Fort Dodge, Iowa). Using a binocular operating microscope, a lateral canthotomy was performed in the right eye and the conjunctiva was incised lateral to the cornea. After separation of the refractor bulbi

muscles, the optic nerve was exposed intraorbitally by blunt dissection. Using calibrated cross-action forceps, the optic nerve was subjected to a crush injury 2 mm from the eye. The uninjured contralateral nerve was left undisturbed. The sciatic nerve was crushed under deep anesthesia, as described previously (26). The sciatic nerve was exposed and a similar crush injury was inflicted, after which the skin was sutured.

Immunocytochemistry

Longitudinal cryosections (20 μm thick) of the nerves were picked up onto gelatin-coated glass slides and frozen until preparation for fluorescence staining. The sections were fixed in ethanol for 10 min at room temperature, washed twice with double-distilled water, and incubated for 3 min in PBS containing 0.05% polyoxyethylene-sorbitan monolaurate (Tween-20). For immunostaining of the cells, coverslips were fixed in methanol for 15 min at −20°C, washed three times with PBS, fixed in acetone for 2 min at room temperature, and again washed three times with PBS. Sections or cells were then incubated for 1 h at room temperature with mouse anti-rat monoclonal antibodies to T cell receptor (TCR) (27), glial fibrillary acidic protein (GFAP) (BioMakor, Rehovot, Israel), FasL (Transduction Laboratories, Lexington, Ky.), ED1 (Serotek, Oxford, U.K.), MHC class II antigens (OX-6) (Serotek, Oxford, U.K.), and cyclic nucleotide phosphohydrolase (CNP) (Promega, Madison, Wis.) or rabbit anti-rat FasL polyclonal antibody (Santa Cruz, Calif.), diluted in PBS containing 3% FCS and 2% BSA. The sections or cells were then washed three times with PBS containing 0.05% Tween-20 and incubated with fluorescein isothiocyanate (FITC)- or Cy3-conjugated goat anti-mouse immunoglobulin G (IgG) (with minimal cross-reaction to rat, human, bovine and horse serum proteins; Jackson ImmunoResearch, West Grove, Pa.) or rhodamine (TRITC) -conjugated goat anti-rabbit IgG (Jackson), for 1 h at room temperature. The sections or cells were washed with PBS containing Tween-20 and treated with glycerol containing 1,4-diazobicyclo-(2, 2, 2) octane to inhibit quenching of fluorescence. The sections and cells were viewed with a Zeiss Universal fluorescence microscope using filters that detect either FITC or Cy3 and TRITC.

Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

Total RNA was isolated from rat optic and sciatic nerves 7 days after injury, as well as from the uninjured nerves and from spleen (using the TRI reagent; Molecular Research Center, Cincinnati, Ohio), according to the manufacturer's instructions. From each sample, 1 μg of total RNA was reverse-transcribed to cDNA using a thermal program of 42°C for 60 min and 95°C for 2 min. Aliquots from each cDNA preparation were amplified by PCR, using the following primers specific for rat FasL and for rat ribosomal protein L19 (RPL19): FasL (forward, 5′-GTTTTTTCTTGTCCATCCTC-3′; reverse, 5′-GCCGCCTTTCTTATACTTC-3′) and RPL19 (forward, 5′-CTGAAGGTCAAAGGGAATGTG-3′; reverse, 5′-GGACAGAGTCTTGATGATCTC-3′), giving a 447 bp and a 194 bp product, respectively. The PCR conditions for rat FasL were 30 s for denaturation at 94°C, 1 min of annealing at 60°C, and 2 min of elongation at 72°C for 35 cycles. The PCR conditions for rat RPL19 were 30 s for denaturation at 94°C, 1 min of annealing at 60°C, and 2 min of elongation at 72°C for 25 cycles. The products were resolved on a 1% agarose gel. The FasL PCR fragment was then isolated from the gel using GenElute agarose spin columns (Supleco, Bellefonte, Pa.) and sequenced.

Immunoblot (Western blot) analysis

Glial cells were extracted with a lysis buffer containing Tris (10 mM, pH 7.5), NaCl (150 mM), Triton X-100 (1%), EDTA (1 mM), spermidine (1 mM), aprotinine (25 μg/ml), leupeptine (25 μg/ml), pepstatin (5 μg/ml), and phenylmethylsulfonylfluoride (PMSF) (1 mM) for 2 h at 4°C with gentle shaking, and the supernatant was collected. For preparation of a high-speed supernatant derived from optic or sciatic nerves, nerves were removed by dissection, frozen in liquid nitrogen, and homogenized immediately in a lysis buffer containing Tris acetate (50 mM), pepstatin (5 μg/ml), leupeptine (25 μg/ml), aprotinine (5 μg/ml), and PMSF (1 mM). The supernatants were collected after high-speed centrifugation (110,000 *g*). Samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (12% gel), followed by blotting onto a nitrocellulose membrane for 2 h at 200 mA (in Tris-glycine). The membrane was incubated overnight at 4°C with PBS containing 5% (v/v) skim milk, incubated with monoclonal antibody to FasL (Transduction Laboratories) in PBS containing 5% skim milk for 1.5 h at room temperature, and washed three times for 20 min in PBS containing 0.05% Tween-20. The membrane was then incubated with horseradish peroxidase-conjugated goat anti-mouse IgG (Sigma) in PBS containing 5% skim milk for 1.5 h at room temperature and washed three times for 20 min in PBS containing 0.05% Tween-20. Immunoreactive bands were visualized by the enhanced chemiluminescence method (ECL, Amersham, U.K.).

In situ detection of cell death by terminal deoxytransferase-catalyzed DNA nick end labeling (TUNEL)

Seven days after crush injury of the optic or sciatic nerves, the rats were killed and their nerves were removed and processed for cryosectioning. Frozen sections were fixed in a 10% formalin solution for 10 min at room temperature and washed twice for 5 min in PBS. The sections were then transferred to 100% methanol for 15 min at −20°C, and washed twice for 5 min with PBS. The samples were rehydrated by serial washings for 5 min in ethanol 100%, 95%, and 70%, and then incubated for 10 min with PBS. For permeabilization, proteases were digested with proteinase K for 20 min at room temperature. Labeling of the ends of the DNA fragments was performed using an *in situ* apoptosis detection kit (Genzyme, Cambridge, Mass.) according to the manufacturer's instructions. The labeled ends were detected using the fluorescein detection kit supplied with a streptavidin-fluorescein conjugate. The fluorescein-stained cells were visualized using a fluorescence microscope.

Analysis of cell numbers in nerve sections

Immunostained cells or TUNEL-reactive cells in each nerve section were counted at the site of injury (discerned by morphology) and at randomly selected areas in the uninjured nerves, using the fluorescence microscope. Each group contained three or four rats. For each nerve, two to four sections were counted and the numbers per mm^2 were calculated and averaged. The results were analyzed using the InStat program. Data were analyzed using one-way analysis of variance, Bartlett's test for homogeneity of variances, and a subsequent Bonferroni multiple comparison *t* test. To detect double-labeled T cells and TUNEL-reactive cells, sections were stained for TUNEL and then immunostained with anti-TCR antibody. Because of high background in the immunostained sections induced by the TUNEL procedure, adjacent sections were also stained for TUNEL or T cells, photographed,

scanned to the computer, analyzed by overlapping images, and documented.

RESULTS

Endogenous T cell accumulation is significantly more pronounced in the injured PNS than in the injured CNS

To compare T cell accumulation at sites of CNS and PNS axonal crush injury, optic and sciatic nerves were excised from rats 3, 7, 14, or 21 days after injury, cryosectioned, and analyzed immunohistochemically for the presence of T cells, using an antibody that recognizes T cells exclusively. Measurement of the numbers of immunolabeled T cells at the injury site revealed a significantly greater accumulation of T cells in the injured sciatic nerve than in the injured optic nerve (**Fig. 1**). In the PNS, large numbers of T cells were detected at the site of the injury and distal to it by day 3. The numbers of T cells reached a peak on day 7 and decreased thereafter. In the CNS, however, T cells arrived in fewer numbers and were localized to a more restricted area around the injury site. As in the PNS, the peak occurred on day 7, after which their numbers decreased.

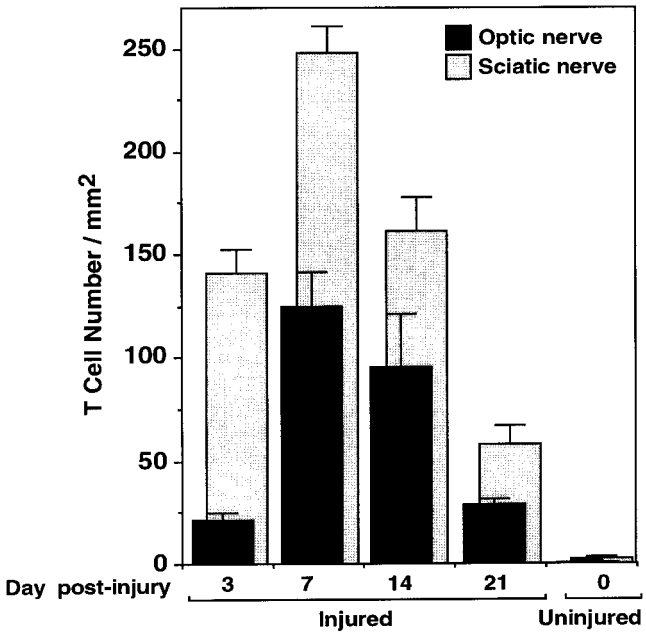


Figure 1. Greater accumulation of endogenous T cells in injured sciatic nerve than in injured optic nerve. At 3, 7, 14, or 21 days after optic or sciatic nerve injury, the injured nerves and the contralateral uninjured nerves were removed, cryosectioned, and analyzed immunohistochemically for the presence of immunolabeled T cells. The histogram shows the mean numbers of T cells ± SE. Statistical analysis (ANOVA) reveals significant differences in T cell numbers between injured optic nerve and injured sciatic nerve on day 3 ($P<0.001$), day 7 ($P<0.001$), and day 14 ($P<0.05$), but not on day 21 ($P>0.05$).

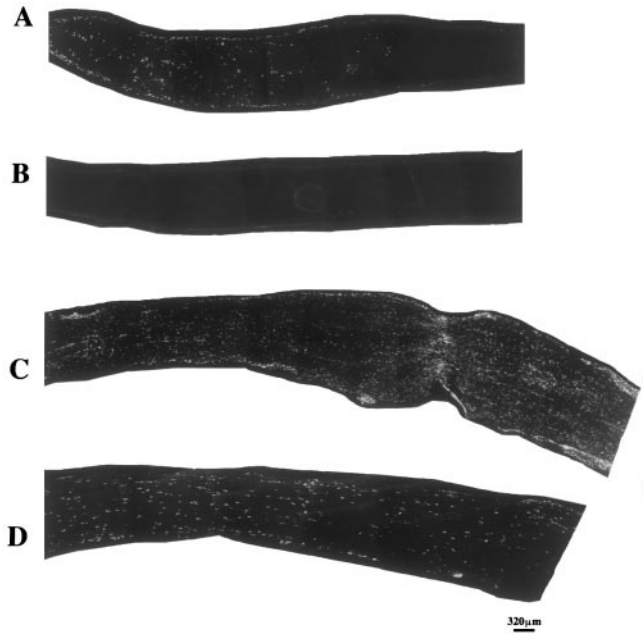


Figure 2. Expression of MHC class II antigens in optic and sciatic nerves. Cryosections taken from injured optic nerve (*A*), uninjured optic nerve (*B*), injured sciatic nerve (*C*), and uninjured sciatic nerve (*D*) were immunostained for MHC class II antigens using OX-6 antibody detected with FITC antibody to mouse IgG. Note the absence of staining in uninjured optic nerve, unlike in uninjured sciatic nerve. After injury, however, staining was detected in the optic nerve and was increased in the sciatic nerve.

MHC class II antigens are constitutively expressed in PNS white matter, but are induced only after injury in CNS white matter

MHC class II molecules on antigen-presenting cells play a key role in presentation of antigens to T cells. We examined uninjured and injured optic and sciatic nerves for MHC class II expression 7 days after crush injury by immunostaining with the OX-6 antibody. MHC class II (Ia) antigens were expressed in the uninjured sciatic nerve, but not in the uninjured optic nerve (**Fig. 2**). Positive immunostaining was observed on scattered interstitial cells. After injury, expression of MHC class II antigens was increased in the sciatic nerve and induced in the optic nerve. Nevertheless, expression of MHC class II antigens in the injured optic nerve was much lower than in the injured sciatic nerve. These results indicate that the white matter of rat PNS constitutively expresses MHC class II antigens, in contrast to rat CNS white matter, where the expression of MHC class II antigens is induced only after injury and is less pronounced.

Death of infiltrating T cells in the CNS 1 wk after injury

To examine the possibility that infiltrating T cells die in the injured CNS, we performed the *in situ* TUNEL procedure, followed by immunohistochemical analysis using anti-TCR antibody. Although TUNEL is used to measure apoptotic cell death, it is reasonable to assume that it might detect necrotic cell death as well, since DNA degradation is an inevitable, albeit late, event in this process. Optic and sciatic nerves were examined 1 wk after crush injury. Extensive T cell infiltration without associated death occurred in the injured sciatic nerve (**Fig. 3**). In contrast, a high level of death among the infiltrating T cells was observed in the injured optic nerve. About 20% of the T cells in the optic nerve and less than 2% in the sciatic nerve were identified as TUNEL reactive. The numbers of TUNEL-reactive cells observed in injured and uninjured optic and sciatic nerves are shown in **Fig. 4**. Whereas more T cells were observed in the sciatic nerve than in the optic nerve after injury, the numbers of TUNEL-reactive cells were higher in the optic nerve. These results suggest that cell death constitutes at least part of the mechanism regulating T cell elimination in the injured CNS.

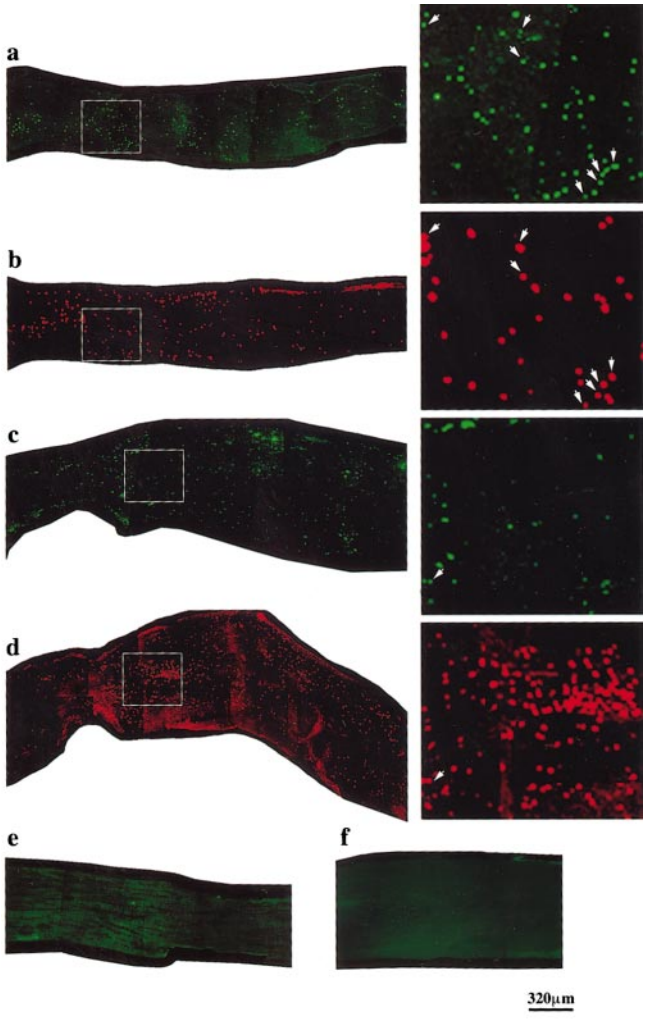


Figure 3. *In situ* detection of TUNEL-reactive cells and immunostained T cells in optic and sciatic nerves. Cryosections from the same injured optic nerve tissue obtained 1 wk after injury were stained for (*a*) TUNEL-reactive cells and (*b*) T cells. Cryosections from the same injured sciatic nerve tissue obtained 1 wk after injury were stained for (*c*) TUNEL-reactive cells, and (*d*) T cells. The arrowheads indicate cells that were double-labeled by TUNEL staining and TCR immunocytochemistry. Cryosections of (*e*) uninjured optic nerve and (*f*) uninjured sciatic nerve stained for TUNEL-reactive cells.

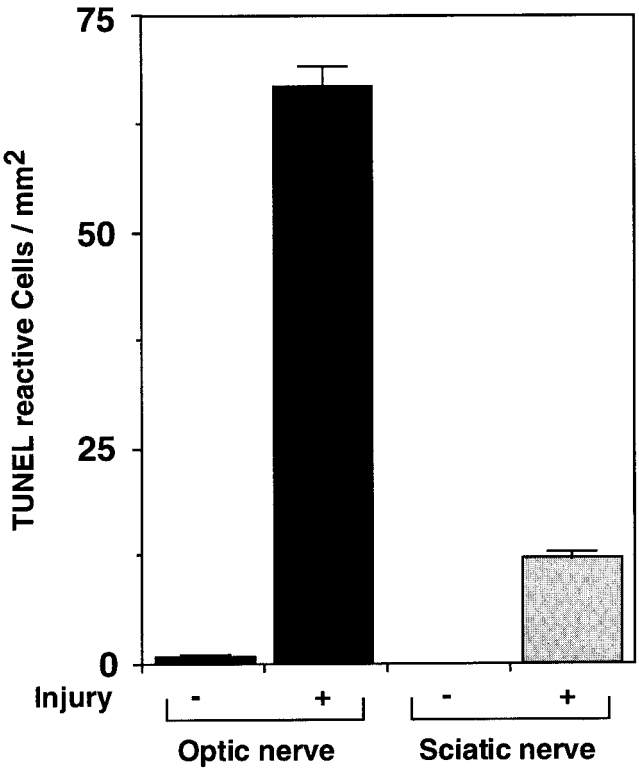
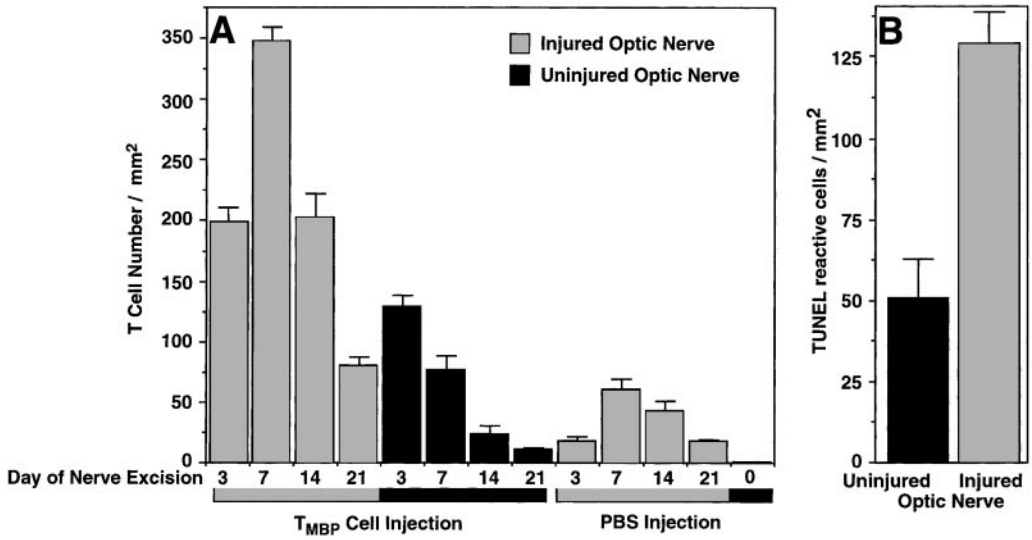


Figure 4. Quantification of TUNEL-reactive cells in injured optic and sciatic nerves. Cryosections were analyzed by TUNEL staining 1 wk after injury. The histogram shows the mean number of TUNEL-reactive cells ± SE. Statistical analysis (ANOVA) revealed significant difference in numbers of TUNEL-reactive cells between injured optic nerve and injured sciatic nerve ($P<0.001$).

T cell accumulation in injured and uninjured CNS is increased after injection of anti-MBP T cells and is accompanied by cell death

To learn whether T cell elimination also occurs in the CNS of rats with EAE, we examined T cell accumulation, disappearance, and death in crush-injured and uninjured optic nerve tissues of Lewis rats injected with anti-MBP T cells. The T cell line used for this experiment (T_{MBP}) can home to and affect the white matter of the CNS (16). The T cell line was activated with MBP for 3 days and then injected i.p. (10×10^6 cells) into rats a few minutes



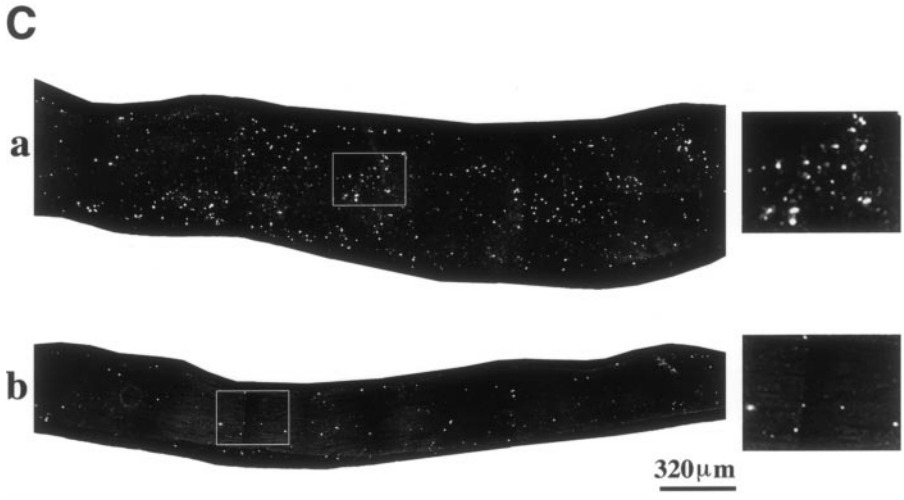


Figure 5. Increased accumulation of T cells in injured and uninjured optic nerves after injection of T_{MBP} cells, accompanied by extensive cell death. *A)* At the time of optic nerve crush, rats were injected i.p. with 10×10^6 activated T_{MBP} cells. Control rats were injected i.p. with PBS. Injured nerves and the contralateral uninjured nerves were removed, cryosectioned, and analyzed immunohistochemically for the presence of immunolabeled T cells. The histogram shows the mean number of T cells ± SE. Statistical analysis (ANOVA) revealed significant differences in T cell numbers between injured optic nerve of T_{MBP}-injected rats and injured optic nerve of control rats on day 3 ($P<0.001$), day 7 ($P<0.001$), day 14 ($P<0.001$), and day 21 ($P<0.01$), and between injured and uninjured optic nerves of T_{MBP}-injected rats on days 3, 7, 14, and 21 ($P<0.001$). *B)* Cell death was assessed by TUNEL staining 1 wk after T_{MBP} cell injection. The histogram shows the mean numbers of TUNEL-reactive cells ± SE. Statistical analysis (ANOVA) revealed significant difference in numbers of TUNEL-reactive cells between injured and uninjured optic nerve ($P<0.001$). *C)* Photomicrographs of (a) injured and (b) uninjured optic nerves of T_{MBP}-injected rats stained for TUNEL.

after injury. The injected rats developed EAE within 3–4 days. Control rats were injected i.p. with PBS. At 3, 7, 14, or 21 days after injury, both the injured and the uninjured optic nerves were excised, cryosectioned, and analyzed immunohistochemically for the presence of immunolabeled T cells. Cell death was assessed by TUNEL in injured and uninjured optic nerves at the peak of T cell accumulation. T cells were detected in the injured optic nerve by day 3, increased to a peak on day 7, and then decreased in number by day 21 (**Fig. 5***A*). At all time points examined, the numbers of T cells detected in the injured optic nerves of T_{MBP}-injected rats were significantly greater than in the injured optic nerves of PBS-injected rats. Fewer T cells were seen in the uninjured nerves of T_{MBP}-injected rats, and their numbers decreased from day 3 to day 21 after the injection. No T cells were detected in uninjured optic nerves of PBS-injected control rats. Although TUNEL-reactive cells were detected in the uninjured optic nerves, their numbers were significantly greater in the injured optic nerves 1 wk after T_{MBP} cell injection (Fig. 5*B, C*), in correlation with the T cell numbers. In both injured and uninjured optic nerves of T_{MBP}-injected rats, ~30% of the T cells were identified as TUNEL reactive (data not shown). During the same period, the number of T cells declined and the animals recovered from the disease. These results suggest that the CNS ability to eliminate T cells does not depend on injury.

Expression of FasL in the white matter of the rat nervous system

Because some tissues appear to require FasL in order to exhibit immune-privileged status by killing infil-

trating lymphocytes and inflammatory cells (28, 29), we examined FasL expression in the rat nervous system. RT-PCR analysis of total RNA isolated from crush-injured and uninjured optic nerves and sciatic nerves showed that FasL mRNA is expressed in both the CNS and the PNS white matter, whether injured or not (**Fig. 6**). The PCR product was sequenced and was found to be homologous to rat FasL.

To examine the expression of FasL protein in the CNS and PNS white matter, we performed immunohistochemical analyses of uninjured sciatic nerves as well as of injured and uninjured optic nerves, using anti-FasL antibody. At the same time, we used anti-ED1 antibody to detect reactive microglia and anti-GFAP antibody to detect astrocytes in the injured optic nerve (**Fig. 7**). Intensive FasL staining, corresponding to the ED1-immunoreactive microglia, was detected at the injury site. Staining was weak in the uninjured optic nerve, and even weaker in the uninjured sciatic nerve (Fig. 7). Different patterns and intensities of FasL staining were observed in the injured and uninjured optic nerves, suggesting differential states of regulation and possibly a different physiological role for FasL under normal conditions compared with trauma.

In an attempt to ascribe FasL immunoreactivity to a particular cell type within the optic nerve as a possible mediator of T cell apoptosis, we analyzed primary cultures of astrocytes, microglia, and oligodendrocytes using anti-FasL antibody and double staining with a specific marker for each cell population. The results pointed to the constitutive expression of FasL in the primary cultures of CNS glial cells (**Fig. 8**). Similar results were obtained by Western blot analysis (**Fig. 9**). Glial cell lysates (astrocytes, microglia, and oligodendrocytes) (Fig. 9*A*) and high-speed supernatants of extracts obtained from injured and uninjured optic nerves (Fig. 9*B*) exhibited intense immunoreactive bands recognized by monoclonal anti-FasL antibody. Weaker bands were detected in high-speed supernatants of uninjured and injured sciatic nerves (Fig. 9*B*). The 40 kDa protein observed in the samples seems to be FasL expressed on the cell membrane. The identity of the upper band at M_r 80 kDa is not known. It might correspond to a soluble form of rat FasL (sFasL), as reported for human FasL (30).

Taken together, these findings confirm the expression of FasL in the white matter of the rat nervous system and demonstrate that all CNS glial cells are capable of expressing FasL protein.

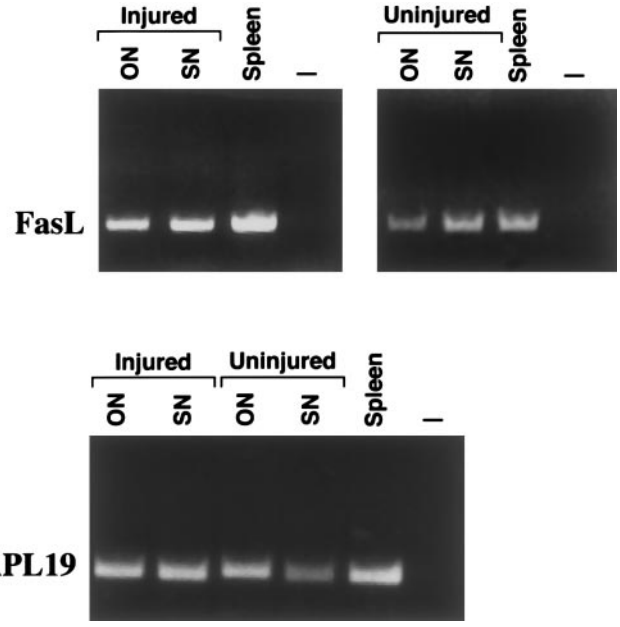


Figure 6. FasL mRNA expression in rat optic and sciatic nerves determined by RT-PCR. RT-PCR analysis was performed on RNA isolated from injured optic nerve (ON), injured sciatic nerve (SN), uninjured optic nerve, uninjured sciatic nerve, and spleen (positive control); each sample was analyzed without the reverse transcriptase enzyme (—) to exclude the possibility of DNA contamination (negative control). FasL was expressed in both rat optic and sciatic nerves, as well as in the spleen. RPL19 was used as a control for the RT-PCR analysis of the isolated RNA.

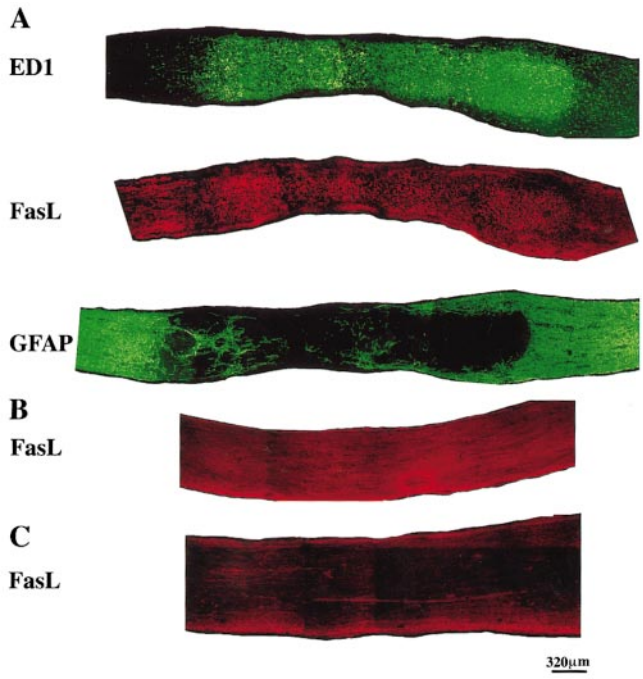


Figure 7. Expression of FasL by optic and sciatic nerves. Cryosections taken from (*A*) injured optic nerve 1 wk after injury, (*B*) uninjured optic nerve, and (*C*) uninjured sciatic nerve were immunostained for FasL using a monoclonal antibody detected with a Cy3 antibody. The same injured optic nerve tissue (*A*) was stained with anti-ED1 antibody to detect activated microglia and macrophages and with anti-GFAP antibody to visualize the injury site (lack of GFAP-positive astrocytes) detected with a FITC antibody. Staining of each of the tissues in the absence of the first antibody was negative.

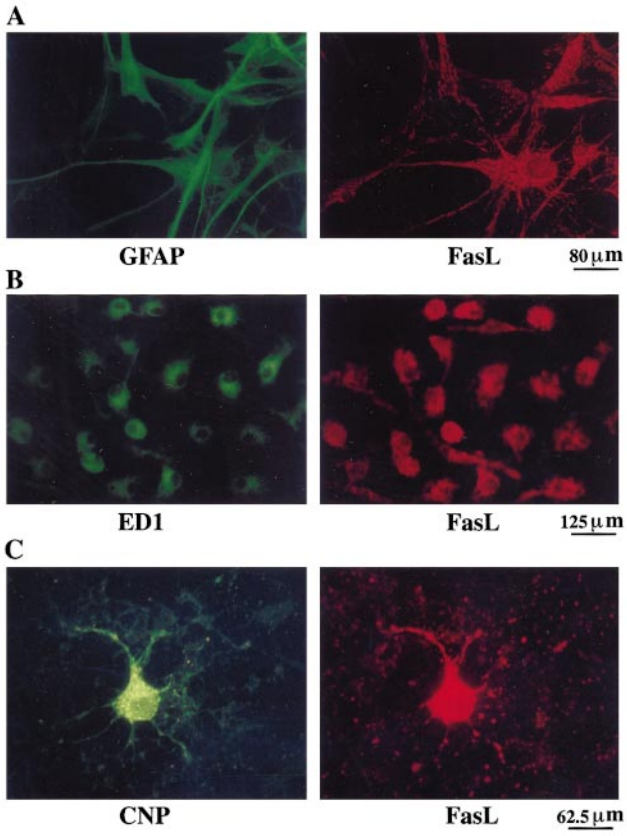


Figure 8. Localization of FasL protein in primary cultures of CNS glial cells by immunohistochemical analysis using polyclonal anti-FasL antibody. Double immunostaining was performed on (*A*) primary cultures of astrocytes using mouse anti-rat GFAP and rabbit anti-rat FasL, (*B*) primary microglial cultures using mouse anti-rat ED1 and rabbit anti-rat FasL, and (*C*) primary oligodendrocyte cultures using mouse anti-rat CNP and rabbit anti-rat FasL. The mouse antibodies were detected using FITC goat anti-mouse IgG and the rabbit antibody was detected using TRITC goat anti-rabbit IgG. In each case, staining of the cells with any of the first mouse antibodies, followed by the second anti-rabbit IgG, or staining with the first rabbit antibody, followed by the second anti-mouse IgG, or staining with the second antibody only, was negative.

DISCUSSION

This study demonstrates that the T cell response to injury in the CNS differs fundamentally from that in the PNS. It has already been established that the CNS, unlike the PNS, is an immune-privileged site (1, 2) and that the immune-privileged status has profound implications for macrophage recruitment and activation after axonal injury (31–33). The mechanism responsible for immune privilege in the CNS is not fully understood, and even less is known about the effects of immune privilege under traumatic conditions. We show that the accumulation of endogenous T cells is significantly greater after PNS trauma than after CNS trauma. Moreover, in contrast to the extensive death of infiltrating T cells in the injured CNS and in rats with EAE, hardly any cell death was detectable in the T cells accumulating in the injured PNS. We further show that MHC class II antigens are expressed by the intact PNS but not by the intact CNS, though their expression in the CNS can be induced by trauma. Finally, we show that FasL is expressed in both CNS and PNS white matter, but more strongly in the CNS.

These findings suggest that T cell-associated immune reactions occur in the CNS white matter after injury and in cases of autoimmune disease. Nevertheless, the T cells appear to be gradually eliminated. Injuries to axons in both the CNS and PNS of mammals result in axonal degeneration distal to the site of the lesion (Wallerian degeneration). However, in contrast to the PNS, axons in the CNS do not regenerate (17). In the present study, a similar course of T cell accumulation was observed after crush injury in the nonregenerative optic nerve and in the regenerative sciatic nerve. In the PNS a few days after injury, large numbers of T cells were seen throughout the nerve, but they disappeared, in temporal correlation with nerve regrowth. In the CNS, however, T cells were recruited in smaller numbers and to a more restricted area around the lesion; they were barely seen distal to the injury site. The decrease in T cell accumulation observed in the CNS from day 7 to day 21 after injury suggests that T cells may be eliminated by regulatory mechanisms. The nature of these accumulated T cells is unknown. However, it was shown in an experimental model of spinal cord injury that T cells isolated from spine-injured rats are capable of causing neurological deficits and histopathological changes similar to EAE when injected intravenously into naive animals. Disease induction was possible only when the T cells were obtained from rats 1 wk postinjury, suggesting that the encephalitogenic T cell repertoire triggered by the injury is under strict regulation (34). Thus, the accumulation of endogenous T cells after CNS axonal injury might be a reflection of a systemic immune response against self components exposed by the injury.

Our observation that death of infiltrating T cells occurs in the CNS after trauma and during spontaneous clinical recovery from EAE, but not in the PNS after trauma, supports the notion of immune privilege in the CNS (1, 12). The similarity in the extent of T cell death detected in injured rat optic nerves and in uninjured optic nerves of T_{MBP}-injected rats suggests that the mechanism of T cell elimination in the CNS is constitutive and is not dependent on injury. Thus, elimination of T cells through cell death appears to play a role in terminating immune reactions in the CNS, but not in the PNS. Similarly, T cell apoptosis was observed in EAE lesions (35–37). In cases of autoimmune disease, the CNS was indeed shown to have a high potential for elimination of T cells through a mechanism of apoptosis that is less

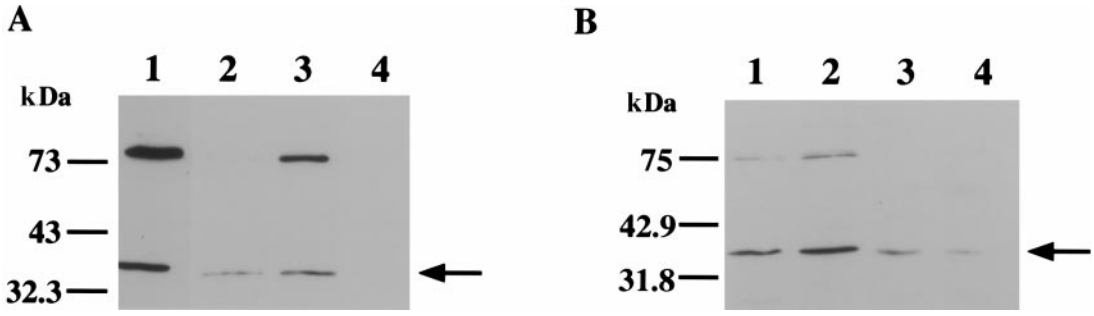


Figure 9. Western blot analysis of FasL expression in primary cultures of CNS glial cells and in optic and sciatic nerve high-speed supernatants. *A*) Qualitative analysis of glial cell lysates. Glial cells were cultured, differentiated *in vitro,* and harvested. Cell lysates of *1)* astrocytes, *2)* microglia, and *3)* oligodendrocytes were prepared and subjected to Western blot analysis using monoclonal anti-FasL antibody, and *4)* each sample was subjected to Western blot analysis using secondary antibody only. *B*) Quantitative analysis of injured and uninjured optic and sciatic nerve high-speed supernatants. High-speed supernatants of homogenized injured and uninjured optic and sciatic nerves were prepared and equal amounts of total protein from each sample of *1)* uninjured optic nerve, *2)* injured optic nerve, *3)* uninjured sciatic nerve, and *4)* injured sciatic nerve were subjected to Western blot analysis using monoclonal anti-FasL antibody. The arrow points to FasL protein in the cell membrane. Protein analysis of all samples with secondary antibody only was negative.

effective in the PNS and almost absent in other tissues such as muscle and skin (38). The present findings do not, however, exclude the possible operation of additional mechanisms of T cell regulation in the CNS after injury and in autoimmune disease. Tolerance of T cells in the CNS may be mediated by anergy or suppression in addition to elimination—for example, by a shift in the reactive T cell population from CD4+ Th1 cells [secreting interferon γ or interleukin 2 (IL-2)] to CD4+ Th2 cells (secreting IL-4 or IL-10), which are capable of suppressing the Th1 inflammatory response. Alternatively, T cell regulation could be controlled by antigen-presenting cells that do not possess the full cohort of secondary signals necessary to activate T cells (e.g., B7 costimulatory molecules). For example, MHC class II+/B7- microglia may ligate the T cell receptor without inducing T cell proliferation. This would result in functional inactivation of the T cells, or anergy (39).

The expression of MHC molecules is an important factor in the process of antigen recognition by T cells. MHC class II molecules are required for antigen presentation to helper T cells. In line with other studies (40, 41), we observed that MHC class II antigens (Ia) are constitutively expressed in the intact PNS but not in the intact CNS. In CNS white matter, MHC class II molecules appear to be inducibly rather than constitutively expressed, i.e., their expression seems to be associated with injury. In the intact peripheral nerve, resident macrophages and fibroblasts are the best candidates to express MHC class II antigens (40, 41). Crushing of the peripheral nerve may also induce these antigens on Schwann cells (42), the main glial element in the PNS. In the CNS, expression of MHC molecules is undetectable immunohistochemically on both oligodendrocytes and neurons (7). Astrocytes and microglia might be the cells expressing MHC class II antigens after central nerve crush injury, as they are induced to express Ia antigens and to function as antigen-presenting cells upon treatment with IFN-γ (9, 43–45). Nevertheless, even after injury, the expression of MHC class II antigens in the CNS is much weaker than in the PNS. This observation further highlights the distinct difference in immunological features between the PNS and the immune-privileged CNS.

The finding that optic nerve expresses FasL protein is in line with reported characteristics of immune-privileged sites and suggests that the Fas-FasL pathway may be involved in inducing death of infiltrating lymphocytes in the CNS, as described in the eye (28) and the testis (29). However, FasL is also expressed, albeit more weakly, in the sciatic nerve, indicating that FasL expression is not unique to immune-privileged sites. Moreover, we were unable to find direct evidence for Fas-mediated T cell cytotoxicity using glial cells expressing FasL. Some cells have indeed been shown to possess very high levels of surface FasL without being cytotoxic (46). Thus, expression of surface FasL may be a necessary but not a sufficient condition for Fas-mediated lysis. In addition, ligation of Fas on freshly isolated T cells has been shown to costimulate cellular activation and proliferation. It thus appears that Fas can mediate opposite effects, depending on the state of T cell activation (47). The differences in intensity and distribution of FasL expression between the injured and the uninjured optic nerve might be attributable to a differential subcellular localization of FasL. This would be in line with a recent report demonstrating that transport of Fas from cytoplasmic stores to the cell surface is an important mechanism in p53-mediated apoptosis (48). Therefore, it is possible that CNS injury and/or autoimmune inflammation can regulate sensitivity to apoptosis by allowing cytoplasmic death receptors to relocate to the cell surface. The observation of FasL expression in the *in vitro* primary cultures of CNS glial cells is consistent

with recent studies showing that FasL is constitutively present in human glial cells *in vivo* (49) and may contribute to the pathogenesis of multiple sclerosis (50).

Although many studies have pointed to a role for FasL in the control of immune responses by induction of apoptosis in infiltrating lymphocytes and granulocytes in the eye (28), testis (29), various murine and human tumors (51–53), and on thyrocytes in patients with Hashimoto's thyroiditis (54), some recent studies have questioned the immunoprotective effect of FasL. Allison et al. (55) reported that expression of FasL in the pancreatic islets of transgenic mice failed to protect these islets against allogenic transplant rejection when placed under the kidneys of recipient mice. The same study demonstrated a proinflammatory function of FasL by induction of a potent granulocytic inflammatory response. Moreover, other recent studies (56, 57) have shown that mice deficient in Fas or FasL are resistant to induction of EAE, and that this is correlated with fewer inflammatory infiltrates and fewer cells undergoing apoptosis in the CNS of the mutant mice. It thus appears that FasL under certain circumstances can mediate apoptosis and under other circumstances can mediate activation and proliferation of immune cells. Taken together, these findings suggest that in the CNS FasL, possibly in conjunction with necessary partner molecules, might help to prevent immune responses by inducing the death of lymphocytes. Other possible functions of FasL, such as regulation of homeostasis or of stress responses, are not excluded. Additional studies are required to evaluate the specific role of FasL in the nervous tissue.

In conclusion, this study demonstrates that the immune-privileged CNS, in contrast to the PNS, uses both passive mechanisms (e.g., reduced expression of MHC class II antigens) and active mechanisms (e.g., death of infiltrating T cells) to limit the T cell immune response after injury and during spontaneous recovery from EAE. This limitation in T cell response may have apparently contradictory effects on the CNS. On the one hand, it prevents the development of massive inflammation and autoimmune diseases in the CNS. On the other hand, however, we have recently showed that increasing the autoimmune T cell response at a site of CNS injury can reduce the secondary degeneration of neurons after a primary axonal injury (58). Thus, immune privilege may be beneficial in protecting the CNS against remodeling of its neuronal network by limiting inflammation, but may be disadvantageous after injury when some immune responses are desirable for CNS recovery. FJ

We thank Shirley Smith and Phil Taylor for editorial assistance, and Igor Friedmann and Haya Avital for help with graphics. Felix Mor generously donated T cell line, Boris Reizis generously donated anti-TCR antibody, and Dvora Teitelbaum generously donated guinea pig MBP. I.R.C. is the incumbent of the Mauerberger Chair in Immunology, Director of the Robert Koch-Minerva Center for Research in Autoimmune Disease, and Director of the Center for the Study of Emerging Diseases. M.S. holds the Maurice and Ilse Katz Professorial Chair in Neuroimmunology. The work was supported in part by the U.S. Army (M.S.), an Alcon award (M.S.), and the Alan Brown Foundation for Spinal Cord Injury (M.S.).

REFERENCES

1. Streilein, J. W. (1995) Unraveling immune privilege. *Science* **270,** 1158–1159
2. Ksander, B. R., and Streilein, J. W. (1994) Regulation of the immune response within privileged sites. *Chem. Immunol.* **58,** 117–145
3. Rao, K., Lund, R. D., Kunz, H. W., and Gill, T. J. D. (1989) The role of MHC and non-MHC antigens in the rejection of intracerebral allogeneic neural grafts. *Transplantation* **48,** 1018–1021
4. Poltorak, M., and Freed, W. J. (1991) BN rats do not reject F344 brain allografts even after systemic sensitization. *Ann. Neurol.* **29,** 377–388
5. Reese, T. S., and Karnovsky, M. J. (1967) Fine structural localization of a blood-brain barrier to exogenous peroxidase. *J. Cell Biol.* **34,** 207–217
6. Barker, C. F., and Billingham, R. E. (1977) Immunologically privileged sites. *Adv. Immunol.* **25,** 1–54
7. Matsumoto, Y., Hara, N., Tanaka, R., and Fujiwara, M. (1986) Immunohistochemical analysis of the rat central nervous system during experimental allergic encephalomyelitis, with special reference to Ia-positive cells with dendritic morphology. *J. Immunol.* **136,** 3668–3676
8. Lassmann, H., Zimprich, F., Vass, K., and Hickey, W. F. (1991) Microglial cells are a component of the perivascular glia limitans. *J. Neurosci. Res.* **28,** 236–243
9. Wucherpfennig, K. W. (1994) Autoimmunity in the central nervous system: mechanisms of antigen presentation and recognition. *Clin. Immunol. Immunopathol.* **72,** 293–306
10. Cserr, H. F., and Knopf, P. M. (1992) Cervical lymphatics, the blood-brain barrier and the immunoreactivity of the brain: a new view. *Immunol. Today* **13,** 507–512
11. Wilbanks, G. A., and Streilein, J. W. (1992) Fluids from immune privileged sites endow macrophages with the capacity to induce antigen-specific immune deviation via a mechanism involving transforming growth factor-beta. *Eur. J. Immunol.* **22,** 1031–1036
12. Streilein, J. W. (1993) Immune privilege as the result of local tissue barriers and immunosuppressive microenvironments. *Curr. Opin. Immunol.* **5,** 428–432
13. Medawar, P. B. (1948) Immunity to homologous grafted skin III. The fate of skin homografts transplanted to the brain, to subcutaneus tissue and to the anterior chamber of the eye. *Br. J. Exp. Pathol.* **29,** 58–69
14. Cserr, H. F., Harling Berg, C. J., and Knopf, P. M. (1992) Drainage of brain extracellular fluid into blood and deep cervical lymph and its immunological significance. *Brain Pathol.* **2,** 269–276
15. Hickey, W. F., Hsu, B. L., and Kimura, H. (1991) T-lymphocyte entry into the central nervous system. *J. Neurosci. Res.* **28,** 254–260
16. Naparstek, Y., Ben Nun, A., Holoshitz, J., Reshef, T., Frenkel, A., Rosenberg, M., and Cohen, I. R. (1983) T lymphocyte lines producing or vaccinating against autoimmune encephalomyelitis (EAE). Functional activation induces peanut agglutinin receptors and accumulation in the brain and thymus of line cells. *Eur. J. Immunol.* **13,** 418–423
17. Perry, V. H., Brown, M. C., and Gordon, S. (1987) The macrophage response to central and peripheral nerve injury. A possible role for macrophages in regeneration. *J. Exp. Med.* **165,** 1218–1223
18. Hirschberg, D. L., Moalem, G., He, J., Mor, F., Cohen, I. R., and Schwartz, M. (1998) Accumulation of passively transferred

primed T cells independently of their antigen specificity following central nervous system trauma. *J. Neuroimmunol.* **89,** 88–96
19. Hirshfeld, H., Teitelbaum, D., Arnon, R., and Sela, M. (1970) Basic encephalitogenic protein: a simplified purification on sulfoethyl-Sephadex. *FEBS Lett.* **7,** 317
20. Ben Nun, A., and Cohen, I. R. (1982) Experimental autoimmune encephalomyelitis (EAE) mediated by T cell lines: process of selection of lines and characterization of the cells. *J. Immunol.* **129,** 303–308
21. McCarthy, K. D., and de Vellis, J. (1980) Preparation of separate astroglial and oligodendroglial cell cultures from rat cerebral tissue. *J. Cell Biol.* **85,** 890–902
22. Raff, M. C., Miller, R. H., and Noble, M. (1983) A glial progenitor cell that develops *in vitro* into an astrocyte or an oligodendrocyte depending on culture medium. *Nature (London)* **303,** 390–396
23. Bottenstein, J. E., and Sato, G. H. (1979) Growth of a rat neuroblastoma cell line in serum-free supplemented medium. *Proc. Natl. Acad. Sci. USA* **76,** 514–517
24. Duvdevani, R., Rosner, M., Belkin, M., Sautter, J., Sabel, B. A., and Schwartz, M. (1990) Graded crush of the rat optic nerve as a brain injury model: combining electrophysiological and behavioral outcome. *Restor. Neurol. Neurosci.* **2,** 31–38
25. Yoles, E., Zalish, M., Lavie, V., Duvdevani, R., Ben-Bassat, S., and Schwartz, M. (1992) GM1 reduces injury-induced metabolic deficits and degeneration in the rat optic nerve. *Invest. Ophthalmol. Vis. Sci.* **33,** 3586–3591
26. Hirschberg, D. L., Yoles, E., Belkin, M., and Schwartz, M. (1994) Inflammation after axonal injury has conflicting consequences for recovery of function: rescue of spared axons is impaired but regeneration is supported. *J. Neuroimmunol.* **50,** 9–16
27. Hunig, T., Wallny, H.-J., Hartley, J. K., Lawetzky, A., and Tiefenthaler, G. (1989) A monoclonal antibody to a constant determinant of the rat T cell antigen receptor that induces T cell activation. *J. Exp. Med.* **169,** 73–86
28. Griffith, T. S., Brunner, T., Fletcher, S. M., Green, D. R., and Ferguson, T. A. (1995) Fas ligand-induced apoptosis as a mechanism of immune privilege. *Science* **270,** 1189–1192
29. Bellgrau, D., Gold, D., Selawry, H., Moore, J., Franzusoff, A., and Duke, R. C. (1995) A role for CD95 ligand in preventing graft rejection. *Nature (London)* **377,** 630–632
30. Tanaka, M., Suda, T., Takahashi, T., and Nagata, S. (1995) Expression of the functional soluble form of human fas ligand in activated lymphocytes. *EMBO J.* **14,** 1129–1135
31. Perry, V., Hume, D., and Gordon, S. (1985) Immunohistochemical localization of macrophages and microglia in the adult and developing brain. *Neuroscience* **15,** 313–326
32. Lazarov-Spiegler, O., Solomon, A. S., Zeev Brann, A. B., Hirschberg, D. L., Lavie, V., and Schwartz, M. (1996) Transplantation of activated macrophages overcomes central nervous system regrowth failure. *FASEB J.* **10,** 1296–1302
33. Hirschberg, D. L., and Schwartz, M. (1995) Macrophage recruitment to acutely injured central nervous system is inhibited by a resident factor: a basis for an immune-brain barrier. *J. Neuroimmunol.* **61,** 89–96
34. Popovich, P. G., Stokes, B. T., and Whitacre, C. C. (1996) Concept of autoimmunity following spinal cord injury: possible roles for T lymphocytes in the traumatized central nervous system. *J. Neurosci. Res.* **45,** 349–363
35. Pender, M. P., Nguyen, K. B., McCombe, P. A., and Kerr, J. F. (1991) Apoptosis in the nervous system in experimental allergic encephalomyelitis. *J. Neurol. Sci* **104,** 81–87
36. Schmied, M., Breitschopf, H., Gold, R., Zischler, H., Rothe, G., Wekerle, H., and Lassmann, H. (1993) Apoptosis of T lymphocytes in experimental autoimmune encephalomyelitis. Evidence for programmed cell death as a mechanism to control inflammation in the brain. *Am. J. Pathol.* **143,** 446–452
37. Smith, T., Schmied, M., Hewson, A. K., Lassmann, H., and Cuzner, M. L. (1996) Apoptosis of T cells and macrophages in the central nervous system of intact and adrenalectomized Lewis rats during experimental allergic encephalomyelitis. *J. Autoimmun.* **9,** 167–174
38. Gold, R., Hartung, H. P., and Lassmann, H. (1997) T-cell apoptosis in autoimmune diseases: termination of inflammation in the nervous system and other sites with specialized immune-defense mechanisms. *Trends Neurosci.* **20,** 399–404
39. Schwartz, R. H. (1989) Acquisition of immunologic self-tolerance. *Cell* **57,** 1073–1081
40. Yu, L. T., Rostami, A., Silvers, W. K., Larossa, D., and Hickey, W. F. (1990) Expression of major histocompatibility complex antigens on inflammatory peripheral nerve lesions. *J. Neuroimmunol.* **30,** 121–128
41. Monaco, S., Gehrmann, J., Raivich, G., and Kreutzberg, G. W. (1992) MHC-positive, ramified macrophages in the normal and injured rat peripheral nervous system. *J. Neurocytol.* **21,** 623–634
42. Bergsteinsdottir, K., Kingston, A., and Jessen, K. R. (1992) Rat Schwann cells can be induced to express major histocompatibility complex class II molecules *in vivo*. *J. Neurocytol.* **21,** 382–390
43. Frei, K., Siepl, C., Groscurth, P., Bodmer, S., Schwerdel, C., and Fontana, A. (1987) Antigen presentation and tumor cytotoxicity by interferon-gamma-treated microglial cells. *Eur. J. Immunol.* **17,** 1271–1278
44. Fierz, W., Endler, B., Reske, K., Wekerle, H., and Fontana, A. (1985) Astrocytes as antigen-presenting cells. I. Induction of Ia antigen expression on astrocytes by T cells via immune interferon and its effect on antigen presentation. *J. Immunol.* **134,** 3785–3793
45. Fontana, A., Fierz, W., and Wekerle, H. (1984) Astrocytes present myelin basic protein to encephalitogenic T-cell lines. *Nature (London)* **307,** 273–276
46. Glass, A., Walsh, C. M., Lynch, D. H., and Clark, W. R. (1996) Regulation of the Fas lytic pathway in cloned CTL. *J. Immunol.* **156,** 3638–3644
47. Lynch, D. H., Ramsdell, F., and Alderson, M. R. (1995) Fas and FasL in the homeostatic regulation of immune responses. *Immunol. Today* **16 (12),** 569–574
48. Bennett, M., Macdonald, K., Chan, S.-W., Luzio, J. P., Simari, R., and Weissberg, P. (1998) Cell surface trafficking of Fas: a rapid mechanism of p53-mediated apoptosis. *Science* **282,** 290–293
49. Bonetti, B., and Raine, C. S. (1997) Multiple sclerosis: oligodendrocytes display cell death-related molecules *in situ* but do not undergo apoptosis. *Ann. Neurol.* **42,** 74–84
50. D'Souza, S. D., Bonetti, B., Balasingam, V., Cashman, N. R., Barker, P. A., Troutt, A. B., Raine, C. S., and Antel, J. P. (1996) Multiple sclerosis: Fas signaling in oligodendrocyte cell death. *J. Exp. Med.* **184,** 2361–2370
51. Hahne, M., Rimoldi, D., Schroter, M., Romero, P., Schreier, M., French, L. E., Schneider, P., Bornand, T., Fontana, A., Lienard, D., Cerottini, J., and Tschopp, J. (1996) Melanoma cell expression of Fas(Apo-1/CD95) ligand: implications for tumor immune escape. *Science* **274,** 1363–1366
52. Niehans, G. A., Brunner, T., Frizelle, S. P., Liston, J. C., Salerno, C. T., Knapp, D. J., Green, D. R., and Kratzke, R. A. (1997) Human lung carcinomas express Fas ligand. *Cancer Res.* **57,** 1007–1012
53. O'Connell, J., O'Sullivan, G. C., Collins, J. K., and Shanahan, F. (1996) The Fas counterattack: Fas-mediated T cell killing by colon cancer cells expressing Fas ligand. *J. Exp. Med.* **184,** 1075–1082
54. Giordano, C., Stassi, G., De Maria, R., Todaro, M., Richiusa, P., Papoff, G., Ruberti, G., Bagnasco, M., Testi, R., and Galluzzo, A. (1997) Potential involvement of Fas and its ligand in the pathogenesis of Hashimoto's thyroiditis. *Science* **275,** 960–963
55. Allison, J., Georgiou, H. M., Strasser, A., and Vaux, D. L. (1997) Transgenic expression of CD95 ligand on islet beta cells induces a granulocytic infiltration but does not confer immune privilege upon islet allografts. *Proc. Natl. Acad. Sci. USA* **94,** 3943–3947
56. Sabelko, K. A., Kelly, K. A., Nahm, M. H., Cross, A. H., and Russell, J. H. (1997) Fas and Fas ligand enhance the pathogenesis of experimental allergic encephalomyelitis, but are not essential for immune privilege in the central nervous system. *J. Immunol.* **159,** 3096–3099
57. Waldner, H., Sobel, R. A., Howard, E., and Kuchroo, V. K. (1997) Fas- and FasL-deficient mice are resistant to induction of autoimmune encephalomyelitis. *J. Immunol.* **159,** 3100–3103
58. Moalem, G., Leibowitz-Amit, R., Yoles, E., Mor, F., Cohen, I. A., and Schwartz, M. (1999) Autoimmune T cells protect neurons from secondary degeneration following central nervous system axotomy. *Nature Med.* **5,** 49–55

Received for publication October 29, 1998.
Revised for publication December 12, 1998.