Protective Autoimmunity Is a Physiological Response to CNS Trauma


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Primary damage caused by injury to the CNS is often followed by delayed degeneration of initially spared neurons. Studies in our laboratory have shown that active or passive immunization with CNS myelin-associated self-antigens can reduce this secondary loss. Here we show, using four experimental paradigms in rodents, that CNS trauma spontaneously evokes a beneficial T cell-dependent immune response, which reduces neuronal loss. (1) Survival of retinal ganglion cells in rats was significantly higher when optic nerve injury was preceded by an unrelated CNS (spinal cord) injury. (2) Locomotor activity of rat hindlimbs (measured in an open field using a locomotor rating scale) after contusive injury of the spinal cord (T8) was significantly better (by three to four score grades) after passive transfer of myelin basic protein (MBP)-activated splenocytes derived from spinaly injured rats than in untreated injured control rats or rats similarly treated with splenocytes from naive animals or with splenocytes from spinaly injured rats activated ex vivo with ovalbumin or without any ex vivo activation. (3) Neuronal survival after optic nerve injury was 40% lower in adult rats devoid of mature T cells (caused by thymectomy at birth) than in normal rats. (4) Retinal ganglion cell survival after optic nerve injury was higher (119 ± 3.7%) in transgenic mice overexpressing a T cell receptor (TcR) for MBP and lower (85 ± 1.3%) in mice overexpressing a T cell receptor for the non-self antigen ovalbumin than in matched wild types. Taken together, the results imply that CNS injury evokes a T cell-dependent neuroprotective response.

Key words: protective autoimmunity; CNS injury; spinal cord injury; optic nerve injury; neuroprotection; degeneration; encephalitogenic T cells

Axonal injury in the CNS initiates processes of retrograde and anterograde degeneration in fibers that suffered direct damage (Aguayo et al., 1991; Villegas-Perez et al., 1993; Peinado-Ramon et al., 1996). In cases of partial injury, the primary insult is followed by a self-propagating process of secondary degeneration, mediated by various toxic agents and resulting in a lateral spread of damage to fibers that had escaped the primary lesion (Ransom et al., 1990; Waxman, 1993; Behrmann et al., 1994; Lynch and Dawson, 1994; Agrawal and Fehlings, 1996; Yoles and Schwartz, 1998a). Pharmacological methods aimed at preventing or slowing down secondary degeneration have included neutralizing the mediators of toxicity, competing with their receptors, or increasing the resistance of spared neurons to the toxic environment (Yoles and Schwartz, 1997, 1998a).

The role of the immune system in the post-traumatic spread of damage has long been a subject of controversy (Hirschberg and Schwartz, 1995; Popovich et al., 1996, 1997, 1998; Cohen and Schwartz, 1999; Schwartz et al., 1999a,b). Anti-inflammatory compounds, such as methylprednisolone (Constantini and Young, 1994) and dexamethasone (Hirschberg and Schwartz, 1995), and anti-inflammatory cytokines (Betha et al., 1999) have been found beneficial in restricting the spread of damage at an early post-traumatic stage; yet, paradoxically, inflammatory cells (macrophages) are needed for regrowth of the injured nerves (Lazarov-Spiegler et al., 1996; Prewitt et al., 1997; Rabchevsky and Streit, 1997; Rapalino et al., 1998). The discovery that macrophages are needed for CNS repair set the stage for a reconsideration of the traditional view of immune activity, and especially autoimmunity, as having only a destructive influence in the context of nerve trauma (Schwartz et al., 1999a,b). Recent studies from our laboratory (Moalem et al., 1999, 2000a;b; Hauben et al., 2000a,b) showed, for example, that autoimmune T cells directed against CNS myelin basic protein (MBP) promoted recovery after crush injury of the optic nerve or contusion of the spinal cord in rats. It was demonstrated that T cells produce neurotrophic factors (Ehrhard et al., 1993; Kerschensteiner et al., 1999) and that the secretion of neurotrophic factors by memory T cells is antigen dependent (Moalem et al., 2000b). It was also shown, however, that injury to the rat spinal cord evokes an autoimmune response directed against myelin and that transfer of MBP-activated lymphocytes from spinaly injured rats causes the monophasic paralytic disease experimental autoimmune encephalomyelitis (EAE) (Popovich et al., 1997, 1998). These apparently conflicting findings raised a key question: does injury awaken only a harmful autoimmune response or also a beneficial response? If injury can indeed trigger a protective immune response, this would imply that injury acts as a stress signal that evokes bene-
ficial autoimmunity in the form of a corrective immune response directed against self (Schwartz, 2000).

In the present study we addressed this key question by conducting three experiments in rats and one in mice. In rats, we first examined whether the outcome of a CNS lesion (in the optic nerve) is better or worse if preceded by a lesion at a different CNS site (spinal cord). Next we examined whether spleen cells transferred from spinally injured rats to newly injured rats exhibit neuroprotective effects. In the third experiment we examined whether the outcome of crush injury to the rat optic nerve, with or without previous contusion of the spinal cord, is worse in rats subjected to thymectomy at birth than in nonthymectomized controls.

The experiment with mice was conducted with transgenic mice overexpressing a T cell receptor (TcR) for MBP (Chen et al., 1996). Using the mouse model of optic nerve injury, we showed previously in our laboratory that active immunization with myelin-associated proteins reduces the rate of retinal ganglion cell (RGC) death (Fisher et al., 2000). This finding prompted us to use the mouse model in the present study to determine whether mice in which >90% of the endogenous T cells bear the receptor for MBP are more resistant to injurious CNS conditions than normal mice.

MATERIALS AND METHODS

Animals

Rats. Adult Sprague Dawley rats (8–10 weeks old, 250–300 gm) were supplied by the Animal Breeding Center of The Hebrew University of Jerusalem. Inbred adult Lewis rats (10–12 weeks old, 200–250 gm) 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 in each experiment.

Mice. Transgenic mice overexpressing a TcR to the CNS self-antigen MBP or to the non-self antigen ovalbumin (OVA) and their matched wild types (B10.PL and BALB/c, respectively) were supplied by Harvard Medical School. The anti-MBP TcR transgenic mice were generated by the injection of rearranged TcR a and b chain constructs derived from an encephalitogenic T cell clone specific for MBP 1–9/IAb. The constructs were injected into C3H/BL/6 fertilized oocytes, and the mice were crossed onto B10.PL mice.

Antigens

MBP was prepared from the spinal cords of guinea pigs (Moalem et al., 2000a,b) or purchased from Sigma (St. Louis, MO). OVA was purchased from Sigma.

Spinal cord contusion

Rats were anesthetized and their spinal cords were exposed by laminectomy at the level of T8. One hour after induction of anesthesia, a 10 gm rod was dropped onto the laminectomized cord from a height of 50 mm, using the NYU impactor (Basso et al., 1995, 1996). Sham-operated rats were laminectomized but not contused.

Preparation of peritoneal exudate cells

Adult rats were injected intraperitoneally with 1 ml of PBS containing 100 µg concanavalin A (Con A; Sigma). Two days later, peritoneal exudate cells (PECs) were harvested by thorough washing of the peritoneal cavity with 40 ml of PBS.

Preparation of activated splenocytes

The rats were killed 3, 7, and 14 d after spinal cord injury, and their spleens were excised and pressed through a fine wire mesh. The washed cells (2 × 10^7/ml) were activated with the antigen (15 µg/ml) and PECs (1 × 10^5/ml) or irradiated thymocytes (2000 rad, 2 × 10^7 cells/ml) in proliferation medium containing 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, and autologous rat serum 1% (v/v). After incubation for 72 hr at 37°C, 90% relative humidity and 7% CO2, cells were washed with PBS, counted, and injected intraperitoneally into autologous rats within 1 hr after spinal cord contusion.

Proliferation assay

Spleen cells from contused rats, excised and pooled 3, 7, and 14 d after spinal cord contusion (n = 3), were cultured in triplicate in flat-bottomed microtiter wells in 0.2 ml of proliferation medium (described above). The cells were cultured in the presence of irradiated thymocytes (2000 rad, 1 × 10^7/ml cells per well) together with MBP (15 µg/ml), OVA (15 µg/ml) or Con A (1.25 µg/ml). The proliferative response was determined by measuring the incorporation of [3H]thymidine (1 µCi per well), which was added for the last 16 hr of a 72 hr culture.

Passive transfer of cells

Within 1 hr of contusion rats were injected intraperitoneally, on a random basis, with spleen cells or with PBS. Sham-operated rats (laminectomized but not contused) were also injected with spleen cells and examined daily for EAE symptoms (Ben-Nun and Cohen, 1982).

In the spinally contused rats, bladder expression was performed manually at least twice a day (particularly during the first 48 hr after injury, when it was done three times a day), until the end of the second week, by which time automatic voidance had been recovered. The rats were carefully monitored for evidence of urinary tract infection or any other sign of systemic disease. During the first week after contusion and in any case of hematuria after this period, they received a course of sulfamerazine (400 mg/ml) and trimethoprim (8 mg/ml) (Resprim, Teva Laboratories, Jerusalem, Israel), administered orally with a tuberculin syringe (0.3 ml of solution per day). Daily inspections included examination of the laminectomy site for evidence of infection and assessment of the hindlimbs for signs of autophagia or pressure.

Assessment of recovery from spinal cord contusion

Behavioral recovery was scored on a scale of 0 (complete paralysis) to 21 (complete mobility) (Behrmann et al., 1994; Basso et al., 1995, 1996, Beattie et al., 1997) by observers blinded to the treatment received by each rat. Approximately once a week, the locomotor activities of the trunk, tail, and hindlimbs were evaluated in an open field by placing each rat for 4 min in the center of a circular enclosure made of molded plastic with a smooth, nonslip floor (90 cm diameter, 7 cm wall height). Before each evaluation, rats were carefully examined for perineal infection, wounds in the hindlimbs, or tail and foot autophagia.

Partial crush injury of the rat optic nerve

The optic nerve was subjected to crush injury as described previously (Yoles and Schwartz, 1998a). Briefly, rats were deeply anesthetized by intraperitoneal injection of Rompun (xylazine, 10 mg/kg; Vitamed, Jerusalem, Israel) and Vetalar (ketamine, 50 mg/kg; Fort Dodge Laboratories, Fort Dodge, IA). With use of a binocular operating microscope, the conjunctiva of the right eye was incised laterally to the cornea. After separation of the retractor 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 1–2 mm from the eye. The left contralateral nerve was left undisturbed.

Measurement of secondary degeneration after crush injury of the rat optic nerve by retrograde labeling of retinal ganglion cells

Secondary degeneration of the optic nerve axons and their attached RGCs was measured after post-injury application of the fluorescent lipophilic dye 4-(4-didecylamino)styril]-N-methylpyridinium iodide (4-Di-Asp) (Molecular Probes Europe BV, Amsterdam, The Netherlands), distally to the lesion site, 2 weeks after crush injury. Application of the dye distally to the lesion site after 2 weeks ensures that only axons that have survived both the primary damage and the secondary degeneration will be counted, because only axons that are intact can transport the dye back to their cell bodies. This approach thus enables us to differentiate between neurons that are still functionally intact and neurons in which the axons are injured but the cell bodies are still viable. With this method, the number of labeled RGCs reliably reflects the number of still-functioning neurons.

Labeling and measurement were performed as follows: the right optic nerve was exposed for the second time, again without damaging the retinal blood supply. Complete axotomy was performed 1–2 mm from the distal border of the injury site, and solid crystals (0.2–0.4 mm...
RESULTS

CNS injury evokes a systemic neuroprotective response

To determine whether the endogenous systemic response awakened by a spinal cord injury in the rat has a beneficial effect, we first examined whether the number of surviving neurons after an optic nerve injury is higher if preceded by a spinal cord injury. A controlled contusive injury was inflicted on the spinal cords of anesthetized male SPD rats by dropping a 10 gm weight from a height of 50 mm onto the laminectomized cord at the level of T8 (Hauben et al., 2000a,b). After 1, 7, 11, 14, 17, 21, or 69 d, the rats (n = 8–14 at each time point) were anesthetized again and subjected to a controlled unilateral partial crush of the optic nerve (Yoles and Schwartz, 1998a). The latter injury was shown previously to serve as a reliable model for the assessment of primary and secondary degeneration and the effects of neuroprotective intervention (Yoles et al., 1992, 1996; Yoles and Schwartz, 1997, 1998a,b; Schwartz and Yoles, 1999). At the same time as the experimental rats underwent spinal cord contusion, control rats were anesthetized but not injured. They were subsequently reanesthetized and subjected to partial crush injury of the optic nerve, as in the experimental rats (n = 9–17; same time points as for the experimental rats). Two weeks after the optic nerve crush, neuronal survival in the injured optic nerve of each rat of both groups was assessed in terms of the number of neurons that had escaped both the initial lesion and the subsequent secondary degeneration. For assessment of surviving axons, a dye was applied distally to the lesion site (see Materials and Methods). Counting of labeled RGCs yielded the number of neurons that were still intact.

In the rats subjected to spinal cord contusion 7, 11, 14, or 17 d before optic nerve crush, the number of surviving optic neurons was significantly higher (by 40% on average) than in rats with crushed optic nerves without previous contusion (Fig. 1). An interval of 1 d between the two lesions was found to be too short for development of an effective response, and ≥21 d appeared to be too long to maintain it. These results suggest that CNS injury evokes a systemic neuroprotective response, detectable here by the improved recovery of a subsequent CNS lesion at another site.

In a similar experiment using female SPD rats, nine rats underw ent spinal cord contusion and seven were sham operated (the spinal cord was exposed and laminectomized without contusion), and after 1 week all 16 rats were subjected to a partial crush injury of the optic nerve. The number of surviving neurons, 2 weeks later, was determined as described above. In the rats with previous spinal injury, the number of labeled RGCs (expressed as a percentage of the mean number of RGCs in the retinas of injured nerves of the sham-operated rats) was 177 ± 36 (mean ± SE). The difference between the two groups was significant (p < 0.05, t test). The use of sham-operated rats rules out the possibility that a beneficial effect is derived from the procedure itself rather than from the response evoked by the damaged neurons.

The systemic beneficial activity evoked by CNS injury is transferable by splenocytes

If the neuroprotective activity elicited by the spinal cord injury is indeed an immune-associated response, in principle it could be mediated either by reactive lymphocytes or by humoral factors such as antibodies, cytokines, or growth factors. In view of previous studies from our laboratory demonstrating that autologous T cells can have a beneficial effect on traumatized CNS neurons (Moalem et al., 1999a, 2000a,b; Hauben et al., 2000a,b),
it was of interest to determine whether lymphocytes transferred from spinal ly injured rats to rats with fresh CNS injuries would be neuroprotective. Successful lymphocyte-mediated transfer of the beneficial immune activity after CNS injury would strongly support the claim that the neuroprotective activity manifested after CNS injury is evoked by T cells.

Splenocytes from the spinal ly injured rats were exposed in vitro for 3 d to MBP together with PECs. The MBP-activated splenocytes were then washed, counted, and administered by systemic injection at a dosage of 4.5 × 10^7 cells to rats newly subjected to spinal cord contusion. The injected lymphocytes had a significant systemic response that can be transferred to recipient rats by systemic neuroprotection evoked by CNS injury in male rats. Male SPD rats were subjected to unilateral partial crush injury of the optic nerve 1–69 d after spinal cord contusion. Two weeks after the crush injury the number of surviving neurons was determined by retrograde labeling of their RGCs. The numbers of rats used at each time point (contused/control) were as follows: day 1, 14/17; day 7, 11/10; day 11, 10/11; day 14, 10/10; day 17, 14/15; day 21, 8/9; day 69, 9/12. At each time point, the ratio between the mean number of RGCs in the retinas of contused rats (i.e., where the optic nerve lesion was preceded by a spinal cord contusion) and the mean number of RGCs in control rats (where the optic nerve lesion was preceded at the corresponding time point by anesthesia only) was calculated. The results are expressed as mean percentages ± SE. The mean numbers of viable fibers (reflected by labeled RGCs) in all the control groups were similar, regardless of the interval between anesthesia and optic nerve crush (50.6, 57.3, 42.53, 58.17, 45.52, 43.25; mean ± SEM for all experiments, 48.85 ± 3.05). The time lapse between the two injuries significantly affected neuronal survival after the optic nerve injury (p < 0.008; ANOVA). Comparison between the groups revealed significantly better neuronal survival when the time lapse between the two injuries was 7–17 d.

Figure 1. Systemic neuroprotection evoked by CNS injury in male rats. Male SPD rats were subjected to unilateral partial crush injury of the optic nerve 1–69 d after spinal cord contusion. Two weeks after the crush injury the number of surviving neurons was determined by retrograde labeling of their RGCs. The numbers of rats used at each time point (contused/control) were as follows: day 1, 14/17; day 7, 11/10; day 11, 10/11; day 14, 10/10; day 17, 14/15; day 21, 8/9; day 69, 9/12. At each time point, the ratio between the mean number of RGCs in the retinas of contused rats (i.e., where the optic nerve lesion was preceded by a spinal cord contusion) and the mean number of RGCs in control rats (where the optic nerve lesion was preceded at the corresponding time point by anesthesia only) was calculated. The results are expressed as mean percentages ± SE. The mean numbers of viable fibers (reflected by labeled RGCs) in all the control groups were similar, regardless of the interval between anesthesia and optic nerve crush (50.6, 57.3, 42.53, 58.17, 45.52, 43.25; mean ± SEM for all experiments, 48.85 ± 3.05). The time lapse between the two injuries significantly affected neuronal survival after the optic nerve injury (p < 0.008; ANOVA). Comparison between the groups revealed significantly better neuronal survival when the time lapse between the two injuries was 7–17 d.
suggest that an MBP-dependent T cell response is evoked physiologically and that its beneficial (neuroprotective) effect can be transferred.

To determine whether the neuroprotective activity can be mediated by anti-MBP antibodies, we collected sera displaying high anti-MBP antibody titers from seven rats and transferred the pooled sera by intravenous injection (2 ml of undiluted sera) to rats newly subjected to spinal cord contusion. No effect was observed on recovery from the spinal cord injury (Fig. 3). We also assayed the anti-MBP antibodies in the sera of spinally contused rats. By 2 weeks after contusion, the presence of antibodies in the recipient rats was barely detectable (data not shown).

In vitro analysis of the T cell response to spinal cord contusion

We measured the proliferative capacity of splenocytes collected from spleens excised 3, 7, and 14 d after spinal cord contusion (Table 1). All proliferative responses were very low. The proliferation of splenocytes from naive rats activated in vitro with MBP (Naive-MBP; n = 5), or OVA (Naive-OVA; n = 5), or splenocytes prepared from sham-operated animals activated with MBP (Naive-MBP; n = 5), or OVA (Naive-OVA; n = 5), were transferred to newly contused rats. A group of newly contused rats injected with PBS (n = 5) was also included. No effect was seen with splenocytes from contused rats incubated in vitro with OVA. Similarly prepared splenocytes, withdrawn from rats 14 d (C) or 3 d (D) after spinal contusion, were activated with irradiated thymocytes in the presence of MBP. After 3 d in vitro the activated spleen cells were collected, washed, and counted. The resuspended cells were injected into six rats newly subjected to spinal contusion (4.5 × 10⁷ cells per rat). In each experiment (C, D), five rats that were subjected to spinal contusion and injected with the vehicle (PBS) served as control. E. Transfer of splenocytes withdrawn 14 d after spinal cord contusion and injected with no previous ex vivo activation into five rats newly subjected to spinal cord contusion. Five rats that were subjected to spinal contusion and injected with the vehicle (PBS) served as control. Behavioral outcome was evaluated according to a double-blind protocol, using a locomotor test with scores ranging from 0 to 21 (see Materials and Methods). Results are expressed as the mean ± SE at each time point tested. Repeated-measures ANOVA revealed significant effect (p < 0.05) in A–C and no effect in D and E.
accumulated T cells at earlier and later post-traumatic stages is cells reminiscent of Th2 helper T cells. The phenotype of the presumably contributing to the observed protection, are perhaps found at the site of the lesion 1 week after contusion, and cord contusion and suggests that among the accumulated T cells confirm that T cells accumulate at the site of injury after spinal using rat RNA was verified as GATA-3 (Fig. 4). These findings the mouse sequence, and the identity of the transcribed product Palmero et al., 1999). Selection of the primers used was based on GATA-3, a Th2-promoting transcription factor (Rodriguez-

Table 1. Numbers of proliferating spleen cells from rats subjected to spinal cord contusion

<table>
<thead>
<tr>
<th>Days after</th>
<th>No antigen</th>
<th>MBP</th>
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<td>contusion</td>
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<tr>
<td>3</td>
<td>1198 ± 150</td>
<td>1121 ± 175</td>
<td>1181 ± 85</td>
<td>11461 ± 3216</td>
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<tr>
<td>7</td>
<td>1208 ± 151</td>
<td>1308 ± 146</td>
<td>1098 ± 66</td>
<td>9308 ± 3694</td>
</tr>
<tr>
<td>14</td>
<td>1109 ± 142</td>
<td>1350 ± 59</td>
<td>1164 ± 21</td>
<td>8994 ± 617</td>
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Spleens from rats with contused spinal cords were excised and pooled. Cell suspensions were prepared from each group, and the numbers of cells were counted. In each well, 1 x 10⁶ cells were stimulated with MBP, OVA, or Con A. Results are expressed in terms of incorporation of [3H]-thymidine (cpm/10⁶ cells). The values shown are means ± SD. The difference between the proliferative responses to MBP and OVA 14 d after the injury is significant (t test, p ≤ 0.05).

Figure 4. Expression of GATA-3 and IL-10, but not of IFNγ, by lymphocytes isolated from contused spinal cords. Lymphocytes were isolated from spinal cords 7 d after spinal cord contusion (SC-L). RNA extracted from the isolated cells was subjected to RT-PCR. RNA extracted from a T cell line directed to MBP [known to express both pro-inflammatory and anti-inflammatory cytokines (Moalem et al., 2006b)] was used as control. The lymphocytes recovered from contused spinal cords expressed mostly IL-10 and hardly any IFNγ.

Figure 5. Adult rats subjected to thymectomy at birth recover poorly from CNS injury. Thymectomized rats (n = 18) were subjected to a partial crush injury of their optic nerves. One week before the optic nerve crush, 8 of these rats underwent spinal cord contusion (precontusion ON crush; thymectomized) and 10 underwent a sham operation (ON crush; thymectomized). Two weeks after optic nerve injury the surviving neurons were labeled, and 5 d after the retinas were excised and their RGCs counted. Normal rats (n = 9) were subjected to optic nerve crush only (ON crush). The number of RGCs per square millimeter in each animal is shown. Thymectomy had a significant effect on RGC survival, both in the absence of previous contusion (p < 0.003) and in the precontused group (p < 0.01; t test).

Lack of endogenous protective autoimmunity in adult rats subjected to thymectomy at birth

To verify that the endogenous protective autoimmune response to injury is part of the body’s own maintenance mechanism, we used adult SPD rats devoid of mature T cells as a result of having undergone thymectomy at birth. On reaching adulthood (2 months), all of the thymectomized rats underwent either spinal cord contusion or a sham operation, and 1 week later all were subjected to a unilateral crush lesion of the optic nerve. Non-thymectomized rats of the same age were subjected to similar optic nerve lesions and used as controls. As shown in Figure 5, counting of labeled RGCs after the injury showed that the number of spared neurons in the thymectomized rats (mean per square millimeter ± SEM, 73 ± 20; n = 10) was significantly smaller than in the nonthymectomized controls (179 ± 25; n = 9, p < 0.01, t test). The number of surviving neurons after an optic nerve injury that was preceded by spinal contusion (94 ± 14; n = 8) was also significantly lower in the thymectomized rats than in the rats with an intact thymus (p < 0.05, t test). No significant differences in the numbers of surviving RGCs were found between the two groups of thymectomized rats, whether or not the optic nerve injury was preceded by spinal contusion. Thus, the beneficial effect of previous contusion, which was observed in rats with an intact thymus, could not be detected in rats lacking mature T cells. These results substantiate our contention that the
systemic response evoked by the spinal cord injury and found to be beneficial after subsequent injury to the optic nerve is mediated by T cells. They further suggest that damaged CNS nerves can indeed benefit from the injury-evoked T cell response but that this response in its natural form does not fully counteract the injury-induced degeneration and requires boosting. Our recent results seem to rule out the possibility that loss of the beneficial systemic response after the thymectomy is caused by nonimmune mechanisms; in rat strains that inherently lack a beneficial response to CNS injury, loss of RGCs in rats devoid of T cells was not higher than in nonthymectomized rats (J. Kipnis, E. Yoles, E. Hauben, I. Shaked, and M. Schwartz, unpublished observations). This difference was borne out by the present results: the RGC survival rate after optic nerve injury was 1617 ± 25 (mean per square millimeter ± SEM) in the wild-type BALB/c mice and 1314 ± 53 (mean per square millimeters ± SEM) in the B10.PL mice. These findings suggest that, compared with the wild type, mice expressing abnormally large numbers of T cell receptors for MBP are better endowed with an endogenous mechanism for coping with injury-induced stress. In contrast, mice lacking the ability to respond to any antigen (including self-antigens), like the transgenic mice overexpressing T cell receptors to OVA (Fig. 6), are defective with respect to their endogenous immune mechanism for coping with the stress imposed by injury.

**DISCUSSION**

The results of this study suggest that beneficial T cell-dependent immunity is a physiological response to CNS trauma and that it partially counteracts the trauma-induced damage.

As shown here, spinal cord injury in the rat evokes a systemic T cell-mediated neuroprotective response, which can be transferred by suitably activated splenocytes into rats with a new spinal injury. In adult rats subjected to thymectomy at birth, recovery from optic nerve injury was worse than the recovery in nonthymectomized rats. It is possible that the same autoimmune T cells (“encephalitogenic” T cells) may be both beneficial for neuronal survival and destructive of myelin, and that what really determines which of these effects will be manifested is the presence or absence of regulatory T cells. Accordingly, the two activities might be mediated by autoimmune T cells that are affected differently by the regulatory T cells common to both activities, or alternatively the presence of these regulatory T cells might make the two effects of the autoimmune T cells mutually exclusive (Nagaoka et al., 2000). The fact that encephalitogenic T cells can be neuroprotective was also recently documented by Hammarberg and his colleagues (2000), who demonstrated that the effects of harmful T cell-derived cytokines such as TNF-α and INF-γ can be curbed by the production of potent neurotrophic factors.

The finding of neuroprotection in transgenic mice overexpressing a T cell receptor for MBP but not in mice overexpressing a T cell receptor for OVA further substantiates the notion that anti-epileptic T cells can indeed benefit from the systemic response evoked by the spinal cord injury and found to be beneficial after subsequent injury to the optic nerve is mediated by T cells. They further suggest that damaged CNS nerves can indeed benefit from the injury-evoked T cell response but that this response in its natural form does not fully counteract the injury-induced degeneration and requires boosting. Our recent results seem to rule out the possibility that loss of the beneficial systemic response after the thymectomy is caused by nonimmune mechanisms; in rat strains that inherently lack a beneficial response to CNS injury, loss of RGCs in rats devoid of T cells was not higher than in nonthymectomized rats (J. Kipnis, E. Yoles, E. Hauben, I. Shaked, and M. Schwartz, unpublished observations).

**Survival rate of retinal ganglion cells after axonal injury is increased in mice overexpressing a T cell receptor for myelin basic protein**

To further substantiate the physiological character of the beneficial T cell-mediated effect specific to self-antigens such as MBP, we assessed the survival rate of RGCs after crush injury to the optic nerves of transgenic mice overexpressing a T cell receptor for MBP. All RGCs in both optic nerves of wild-type and transgenic mice were stereotactically prelabeled. After 3 d the left optic nerve of each mouse was severely crushed, and the retinas of the eyes were subjected to unilateral crush injury. After 1 week the retinas were excised and whole mounted, and their labeled (surviving) RGCs were counted. The mean numbers of RGCs ± SEM in each group of transgenic mice are expressed here as percentages of the matched wild type. Survival of the injured mice overexpressing a T cell receptor for MBP (n = 10) was significantly higher than in the corresponding wild type (n = 10; p < 0.009), whereas in mice overexpressing a T cell receptor for OVA (n = 9) survival was lower than in the corresponding wild type (n = 10; p < 0.0003; t test).

**Figure 6.** Survival of retinal ganglion cells after optic nerve crush in transgenic mice overexpressing a receptor for anti-MBP T cells or anti-OVA T cells. All RGCs of both eyes of transgenic mice overexpressing a T cell receptor for MBP or a T cell receptor for OVA and of the corresponding wild-type mice were labeled with a stereotactically injected dye. Three days later the mice were subjected to a unilateral crush injury. After 1 week the retinas were excised and whole mounted, and their labeled (surviving) RGCs were counted. The mean numbers of RGCs ± SEM in each group of transgenic mice are expressed here as percentages of the matched wild type. Survival of the injured mice overexpressing a T cell receptor for MBP (n = 10) was significantly higher than in the corresponding wild type (n = 10; p < 0.009), whereas in mice overexpressing a T cell receptor for OVA (n = 9) survival was lower than in the corresponding wild type (n = 10; p < 0.0003; t test).

Table 1 shows the results of this experiment. The number of surviving RGCs per square millimeter after optic nerve crush injury (1367 ± 47, n = 9) was significantly lower (p < 0.0003) than in the wild type (1617 ± 25, n = 10). It should also be noted that the genetic backgrounds of the transgenic mice selected for overexpression of the T cell receptor for MBP and OVA were different (B10.PL and BALB/c, respectively). B10.PL is a mouse strain that shows a low recovery rate after optic nerve injury and is amenable to immunological boosting, whereas BALB/c mice are characterized by a high survival rate after optic nerve injury (Kipnis, Yoles, Hauben, Shaked, and Schwartz, unpublished observations). This difference was borne out by the present results: the RGC survival rate after optic nerve injury was 1617 ± 25 (mean per square millimeter ± SEM) in the wild-type BALB/c mice and 1314 ± 53 (mean per square millimeters ± SEM) in the B10.PL mice. These findings suggest that, compared with the wild type, mice expressing abnormally large numbers of T cell receptors for MBP are better endowed with an endogenous mechanism for coping with injury-induced stress. In contrast, mice lacking the ability to respond to any antigen (including self-antigens), like the transgenic mice overexpressing T cell receptors to OVA (Fig. 6), are defective with respect to their endogenous immune mechanism for coping with the stress imposed by injury.
On the basis of the present study, it seems reasonable to suggest that autoimmune T cells may have a beneficial effect in the context of CNS injury. It was not clear, however, whether the observed autoimmune neuroprotection represents a physiological response to injury or was the result of an exogenous application of autoimmune T cells. The present work demonstrates that the T cell-mediated neuroprotection is likely to be a purposeful physiological response. It further demonstrates that this response reaches a peak within 7 d and is maintained for an additional 10 d, after which it disappears. The fact that the recovery from optic nerve injury was worse in thymectomized rats than in rats with an intact thymus suggests that the latter group derives at least some benefit from an endogenous T cell-related immunity, which evidently is amenable to therapeutic boosting. In rat strains that are inherently incapable of sustaining a beneficial response to injury, the absence of mature T cells (caused by thymectomy) did not worsen their recovery (Kipnis, Yoles, Hauben, Shaked, and Schwartz, unpublished observations). Other recent studies have also indicated that adaptive immunity plays a role in regulating the survival of neurons after nerve injury. Thus, for example, the neuronal loss after facial nerve injury was greater in mice deficient in functional T and B cells than in their wild-type controls (Serpe et al., 1999).

In a recent study from our laboratory, expression of the co-stimulatory molecule B7.2 in rats was found to be increased after spinal contusion and was further increased after passive transfer of autoimmune T cells that led to neuroprotection (Butovsky et al., 2001). In view of the recently discovered close relationship between Th2 expression and expression of the B7.2 receptor CD28 (Rodriguez-Palmero et al., 1999), it is possible that the T cells that were isolated from the contused spinal cord 1 week after contusion and express features reminiscent of Th2 cells, represent one of the T cell populations participating in the multicellular process of neuroprotection. These populations might include both encephalitogenic T cells and regulatory T cells, and the presence of the one without the other may not be sufficient for neuroprotection (Schwartz and Kipnis, 2001).

This is the first demonstration, confirmed by four independent experimental approaches, that a T cell-dependent autoimmune process is evoked for the benefit of the individual. This finding may explain the frequently described presence of T cells directed to self-antigens (such as MBP) in healthy individuals, which in turn leads to the speculation that autoimmune T cells may in some cases be harmless or even useful (Cohen, 1992; Matzinger, 1994). On the basis of the present study, it seems reasonable to suggest that the traumatic event acts as a stress signal to the immune system, with the purpose of helping the damaged nerve cope with the threat of progressive degeneration, a potentially devastating event in the CNS. The spontaneous response, although beneficial if tightly regulated, may nevertheless be insufficiently effective, possibly because of the immune-privileged character of the CNS (Cohen and Schwartz, 1999; Schwartz et al., 1999a). These results support our contention that autoimmune protection, being the body’s own physiological (although inadequate) response to injury, is worth boosting for therapeutic purposes.

REFERENCES


