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Review article

The role of T helper cells in neuroprotection and regeneration

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Abstract

The inflammatory wound healing response of the central nervous system (CNS) following mechanical injury is characterized by at least one or two phases of T cell infiltration. Surprisingly, whether T cells play a beneficial or detrimental role in these processes is still controversial. It has been suggested that autoimmune T cells may provide "protective autoimmunity", however, after CNS injury, injections of autoimmune T cells and vaccine strategies led to both improvement in some models and exacerbation of the damage in others. Here, we review increasing evidence that a specific T cell subpopulation, namely T helper cells type 2 (Th2 cells) are particularly beneficial in the context of CNS lesions. CNS injuries such as mechanical lesions or stroke induce a systemic immunosuppression, which is characterized by a systemic shift towards a Th2 cytokine pattern. Simplified, this systemic Th2 shift results in reduced cell-mediated immune responses, and, to a lesser extent, humoral immune responses. Furthermore, treatment with potent Th2 inducers such as glatiramer acetate or statins, as well as vaccination strategies using Th2-inducing adjuvants for immunization such as aluminum hydroxide, result in increased neuroprotection and regeneration — without development of autoimmune CNS inflammation. Thus, it is tempting to speculate that a systemic Th2 shift is part of a necessary CNS wound healing response after injury, by furthering regeneration and preventing autoimmune disease of the CNS. Within this context, investigating the potential of a systemic Th2 shift to improve outcome after CNS injury, including the control of possible side-effects such as increased susceptibility to infection and allergic responses, is extremely promising.
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Keywords: Th2; Protective autoimmunity; Immunosuppression; CNS wound healing; Traumatic brain injury; Spinal cord injury

Contents

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1. Introduction

Mechanical injury of the CNS is followed by an inflammatory wound healing response, including at least one or two phases of T cell infiltration. Here, we review the contradictory results regarding the beneficial or detrimental effects of T helper cells in the treatment of traumatic CNS damage and summarize as yet unanswered questions in relation to the particularly beneficial role of a specific T cell subpopulation, namely T helper cells type 2 (Th2 cells), in the context of CNS lesions.

2. The wound healing response of the central nervous system

2.1. CNS wound healing versus autoimmune diseases

To understand the conflicting reports on T cell effects in the CNS during pathological processes it is crucial to differentiate between autoimmune processes and the physiological wound healing response of the CNS following mechanical damage. During autoimmune processes such as multiple sclerosis, T cells represent major causative agents of the disease. In contrast, after mechanical damage such as spinal cord injury or traumatic brain injury, T cells are key players in the wound healing response (Schwartz, 2005).

2.2. T cells in the CNS

The CNS is routinely and effectively surveyed by the immune system (Engelhardt and Ransohoff, 2005). Nearly two decades ago, it has been demonstrated that activated lymphocytes enter the CNS in the absence of overt inflammatory disease (Hickey et al., 1991; Wekerle et al., 1987). In the healthy brain only low numbers of T lymphocytes are present (Hickey, 1999), which are elevated during a strong immunological response in the body, even if the nervous system is itself not involved (Hickey and Kimura, 1987), suggesting that the immunosurveillance of the entire body including the CNS is increased after immunological challenge. Human central memory CD4+ T cells enter the cerebrospinal fluid, across either the choroids plexus veins or the meningeal blood vessels, to monitor the subarachnoid space, retaining the capacity to either initiate local immune reactions or return to secondary lymphoid organs (Kivisakk et al., 2003).

2.3. T cell infiltration after CNS injury

Activated T cells seem to accumulate at the CNS lesion site irrespective of their antigen specificity. Within hours after injection, activated T cells enter the healthy CNS parenchyma and the CNS-specific cells either remain there or cyclically reenter the brain (Hickey et al., 1991). In Lewis rats with unilaterally injured optic nerves, systemically administered T cells specific either for the self antigen myelin basic protein (MBP) or specific for the non-self antigen ovalbumin (OVA) accumulate in the injured optic nerve but not in the healthy control side (Hirschberg et al., 1998; Moalem et al., 1999b). Approximately 24 h after mechanical lesion increased numbers of T cells infiltrate the brain or the spinal cord of rodents, and remain for at least 7–14 days, depending on the model (Babcock et al., 2003; Schnell et al., 1999; Hendrix and Kramer, unpublished observation). After entorhinal cortex lesion, low numbers of invading CD4+ T cells were consistently found in the zone of anterograde degeneration (Fig. 1; Bechmann et al., 2001). A second, late phase of T cell infiltration after spinal cord

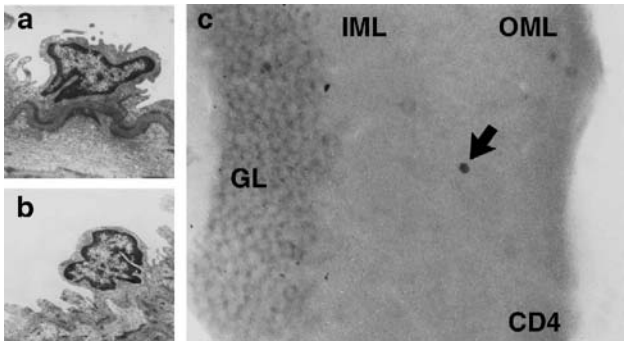

Fig. 1. T cell infiltration after entorhinal cortex lesion. After lesion, homing T cells were found in the zone of anterograde degeneration at the ultrastructural level (a, b). Only single CD4-positive cells were found in the zone of anterograde degeneration (arrow in panel c) at any timepoint investigated after lesion. IML: inner molecular layer, OML: outer molecular layer, GL: granular cell layer. (Reproduced with written permission from Bechmann et al., 2001).

injury has been reported in mice exposed to mouse hepatitis virus in a conventional breeding facility, while this late-phase response was absent in mice kept in specific pathogen-free facilities (Schnell et al., 1997). Since murine hepatitis virus is endemic in wild mice, it should be considered that specific pathogen-free mice represent an artificially clean population and that the second phase of T cell infiltration plays an important part in the physiological wound healing response.

In summary, T cells infiltrate and accumulate in the CNS after lesion. Interestingly, there is evidence that CNS injury is associated with the presence of activated autoimmune T cells.

3. The controversial role of T cells in CNS wound healing

3.1. Autoreactive T cells after CNS injury

Activated CNS-reactive T cells are considered to be key players in the induction and maintenance of autoimmune diseases such as multiple sclerosis. However, they are also activated in spinal cord injury (Jones et al., 2002; Popovich et al., 1996) and after experimental and clinical nerve trauma (Olsson et al., 1992, 1993), which are considered to be non-autoimmune conditions. Furthermore, clinical studies have reported increased numbers of MBP-reactive T cells in spinal cord injury and stroke patients (Kil et al., 1999; Wang et al., 1992).

Seven days after spinal cord injury in the rat, autoreactive T cells can be isolated from peripheral lymph nodes. These T cells react with myelin basic protein (MBP), an important protein of the myelin sheath. These CNS-reactive T cells can exacerbate axonal injury, demyelination and functional loss after spinal cord injury in Lewis rats and transgenic mice with high levels of MBP-reactive T cells (Jones et al., 2002; Popovich et al., 1996). When these autoreactive T cells are injected into healthy animals, they develop a phenotype reminiscent of experimental autoimmune encephalomyelitis (EAE) (Popovich et al., 1996). T cells isolated after this timepoint do not induce similar symptoms, suggesting regulatory mechanisms that suppress autoimmune reactions (Popovich et al., 1996). However, there is considerable debate as to whether these autoimmune T cells contribute to neurodestruction and demyelination during this transient phase of T cell activation or whether they exert neuroprotective effects and support neuroregeneration (Popovich and Jones, 2003; Schwartz, 2005).

3.2. CNS injury in T cell-deficient mice

The analysis of T cell-deficient mice with and without replenishing T cells seems a feasible step to further understanding the effects of lymphocytes on CNS. Fee et al. (2003) reported that aseptic cerebral injury is attenuated in T cell-deficient RAG1(−/−) mice and replenishment of activated CD4 T cells exacerbated cerebral injury in RAG1(−/−) mice (Fee et al., 2003). In contrast, after experimental axotomy of the facial nerve of immunodeficient SCID mice, the survival of facial motor neurons was severely impaired compared to immunocompetent wild-type mice. Reconstitution of SCID mice with wild-type splenocytes containing T and B cells restored the survival of facial motor neurons in these mice to the level of the wild-type controls (Serpe et al., 1999).

Thus, in one model, replenishment of T cells exacerbated the injury and in another model it increased cell survival. It is important to note that these studies are difficult to compare for a number of reasons. The mouse lines display profound differences in their immune systems, the lesion is either in the CNS or in the peripheral nervous system (PNS), the parameters evaluated are different, and either activated CD4+ T cells or mixed splenocytes have been administered without further characterization of the T cell subtypes. Thus, further studies are needed to detect those lymphocyte subpopulations that exert either beneficial or detrimental effects in defined pathophysiological contexts such as spinal cord injury and traumatic brain injury.

3.3. Protective autoimmunity

In contrast to the general notion that T cell infiltration is primarily harmful to the CNS, there is increasing evidence that T cells may also beneficially influence lesion development following CNS trauma. Michal Schwartz's group has proposed the concept of "protective autoimmunity", whereby autoreactive T cells directed to specific self-antigens are recognized as "the physiologic fighting force against acute and chronic neurodegenerative conditions" (Moalem et al., 1999a; Schwartz, 2005). In their landmark publication, Moalem et al. (1999a) reported that injection of activated anti-MBP T cells protects retinal ganglion cells after partial optic nerve crush in rats. In this model, activated rat T cells specific for MBP, heatshock protein (HSP) and OVA were injected intraperitoneally into rats. All types of activated T cells accumulated in the injured optic nerve but not in the healthy contralateral optic nerve (Hirschberg et al., 1998; Moalem et al., 1999a). Using a neurotracer the degree of primary and secondary damage to optic nerve axons and their attached retinal ganglion cells was analyzed immediately after the injury, and again after two weeks. The percentage of labeled retinal ganglion cells reflects the viable axons, which are still able to transport the neurotracer. Only autoreactive MBP-specific T cells were able to protect retinal ganglion cells from secondary damage — in contrast to T cells specific for the non-self antigen OVA or T cells specific for HSP, which is not restricted to the CNS. Thus, the accumulation of activated T cells in the lesion site appears to be antigen-*independent*, while neuroprotection appears to be a characteristic of T cells specific for CNS antigens.

In a multitude of follow-up studies published, Michal Schwartz's group extended the concept of "protective autoimmunity" to many other CNS and PNS lesion paradigms (Table 1). Several other laboratories have reported protective and/or pro-regenerative effects of T cells or vaccine strategies

Table 1
Selected studies on neuroprotective or pro-regenerative effects of T cells

Model/lesion type	Species	Treatment; Pre-/post-le sional?	Th1/Th2? Autoreactive?	Results	EAE?	Reference
Partial optic nerve crush	Rat, adult female Lewis	Immediate post-lesional injection of activated anti-MBP T cells after optic nerve injury	Th1, autoreactive	Increased survival of retinal ganglion cells (RGC); heat shock protein (hsp60)- or OVA-specific T cells did 'home' to the site of injury but did not protect!	Yes	Moalem et al. (1999a)
Partial optic nerve crush	Mouse, adult female C3H.SW and SJL/J	Pre-lesional immunization with encephalitogenic or nonencephalitogenic peptides of proteolipid protein (PLP) or myelin oligodendrocyte glycoprotein (MOG) in CFA	Th1, autoreactive	Protection by nonencephalitogenic peptides pPLP 190–209 or pMOG 1–22 significantly higher than by OVA or a beta-amyloid-derived peptide; protection by encephalitogenic pPLP 139–151 only when mild (and not strong) EAE was induced	No/ Yes	Fisher et al. (2001)
Small dorsal hemisection of the corticospinal tract	Mouse, adult female BALB/c	Pre-lesional immunization 2×/week with homogenate of spinal cord in IFA	Th2, autoreactive	Long-distance regeneration of CST fibers, functional recovery (contact-placing test)	No	Huang et al. (1999)
Large dorsal hemisection of the corticospinal tract	Mouse, adult female SJL/J (susceptible to EAE)	Pre-lesional immunization with myelin or with a cocktail of rN ogo66/rMAG in IFA or Alum	Th2, autoreactive	Long-distance axon regeneration in all types of immunization. Better after myelin immunization. Alum was as or more effective than IFA	No	Sicotte et al. (2003)
Spinal cord contusion	Rat, adult female Lewis	Post-lesional (1 h) transfer of T cells and pre-lesional immunization with MBP in IFA	Th2, autoreactive	Improved functional recovery (BBB score)	Yes	Hauben et al. (2000a, b)
Spinal cord contusion	Rat, adult female Lewis	Immediate post-lesional transfer of MBP-specific T cells; pre-lesional immunization with MBP in CFA or IFA	Th1 = Th2 > PBS	Varying degrees of functional impairment (BBB score), exacerbated lesion pathology, greater rubrospinal neuron loss, increased intraspinal T-cell accumulation, and enhanced macrophage activation relative to SCI control groups; final BBB scores were different, the frequency of weight support and plantar stepping was greater in rats injected with adjuvant (CFA or IFA) compared to PBS. No differences between CFA- and IFA-injected rats	Yes/ No	Jones et al. (2004)
Spinal cord contusion	Rat, adult Lewis or Sprague-Dawley	Pre- and post-lesional immunization with MBP-derived altered peptides in IFA or CFA with high or low dose of M. tuberculosis	Th1 > Th2 (no systematic comparison) autoreactive	Improved functional recovery (BBB score); higher preservation of longitudinally ordered anisotropy) — CFA induces an earlier and stronger T cell response than that induced by IFA. EAE-resistant SPD rats show better spontaneous recovery than susceptible Lewis rats; loss of the beneficial effect, together with signs of severe encephalitogenicity when the bacterial dosage was high	Yes	Hauben et al. (2001a)
Spinal cord contusion; partial optic nerve crush	Rat, adult Sprague-Dawley and Lewis	Post-lesional immunization with Nogo-A-derived peptide (p472) in CFA; passive transfer of T cell	Th1, autoreactive	Improved functional recovery (BBB score) after SCI; increased RGC survival after optic nerve crush	No	Hauben et al. (2001b)

(continued on next page)

Table 1 (*continued*)

Model/lesion type	Species	Treatment; Pre-/post-le sional?	Th1/Th2? Autoreactive?	Results	EAE?	Reference
		line against the Nogo-A peptide				
Spinal cord contusion	Mouse, B10.PL+Vβ4/Vβ8.2 MBP TCR Tg	Use of transgenic mice in which >95% of all CD4+T-lymphocytes are reactive with myelin basic protein (MBP)	–	Significantly impaired recovery of locomotor and reflex function in Tg mice compared with non-Tg littermates; significantly less white matter at the injury site; increased rostrocaudal lesion expansion (*i.e.*, secondary degeneration) in Tg mice.	–	Jones et al. (2002)
Ventral root avulsion	Rat, LEW.RT1$_{L}$, LEW.RT1$_{AV1}$, and DA.RT1$_{AV1}$	Pre-lesional immunization with an encephalitogenic myelin basic protein peptide in CFA	Th1, autoreactive	Reduction of spinal motoneuron loss	Yes	Hammarberg et al. (2000)
Aseptic cerebral injury	Mouse, C57BL/6; RAG1(−/−) mice	Adoptive transfer of CD4(+)CD62L(low)CD44(high) activated/effector T cells 24 h prior to ACI	Not defined	ACI is attenuated in RAG1(−/−) mice compared to C57BL/6 animals. Adoptive transfer of CD4(+)CD62L(low)CD44(high) activated/effector T cells 24 h prior to ACI into RAG1(−/−) mice resulted in a significantly enhanced acute ACI that was comparable to ACI in the C57BL/6 animals. Adoptive transfer of CD 4(+)CD62L(high)CD44(low) naive/non-activated T cells did not increase ACI in the brains of RAG1(−/−) mice. T cell inhibitory agents, cyclosporin A (CsA) and FK506, significantly decreased ACI-induced acute damage in C57BL/6 mice.	No	Fee et al. (2003)
Aseptic cerebral injury (nitrogen-chilled steel rod held for 6s against intact scull)	Mouse, female C57BL/6	Pre-lesional immunization with MOG either in CFA or IFA	Th1=Th2 MOG=OVA Autoreactive=non-autoreactive	Accelerated revascularization and improved "healing index" (cellular infiltration, necrosis, vascularity — neuronal damage not clearly defined in this index!) by all IFA or CFA-associated MOG- or OVA-specific T cells	No	Hofstetter et al. (2003)
Closed head injury	Mouse, male adult C57BL/6J and Balb/c/OLA	Post-lesional vaccination with glatiramer acetate (GA) in CFA	Th1, autoreactive	Strain differences+correlation of recovery with resistance to CNS autoimmune disease; GA vaccination improves functional recovery	No	Kipnis et al. (2003)
Ischemic stroke after middle cerebral artery occlusion (MCAO)	Mouse, female C57BL/6	Nasal vaccination by myelin oligodendrocyte glycoprotein (MOG) (35–55) peptide; adoptive transfer of MOG-specific T cells	Th1, autoreactive	Nasal MOG was most efficacious and reduced ischemic infarct size by 70% at 24 h as well as improving behavior score. Adoptive transfer of CD4+ T cells from nasally tolerized mice to untreated mice prior to MCAO surgery significantly decreased stroke size; CD4+ T cells from nasally tolerized IL-10-deficient mice had no significant effect.	No	Frenkel et al. (2003, 2005)
Glaucoma (laser-induced blockage of aqueous outflow)	Rat, adult male Lewis and Sprague-Dawley	Cop-1 immunization in CFA or without adjuvant; topical Cop-1 administration as eye drops	Th1/?, autoreactive	Increased survival of RGCs	No	Bakalash et al. (2005)
Unilateral facial nerve axotomy; amyotrophic lateral sclerosis (SOD-1 mice)	Mouse, adult female C57BL/6JO1aHsd	Cop-1 immunization in CFA	Th1, autoreactive	Increased survival of motoneurons; functional recovery; prolonged life-span of SOD-1 mice	No	Angelov et al. (2003)

Axotomy of the facial nerve	Mouse, C.B-17 (1/1) wild-type and C.B-17 (2/2) scid; RAG-2 KO (Balb/c), and Balb/c	Reconstitution of immunodeficient SCID or RAG-2 KO mice with wild-type splenocytes containing T and B cells	–	Reduced survival of facial motor neurons (FMN) after facial nerve axotomy in SCID or RAG-2 KO mice compared to wild-type mice. Reconstitution restores FMN survival and improved functional recovery (full eye blink reflex, vibrissae movements)	–	Serpe et al. (1999, 2000, 2002)
Axotomy of the facial nerve	Mouse, female C57BL/6, CD4 KO or RAG-2 KO	Reconstitution of CD4 KO or RAG-2 KO mice with CD4+ T cells	–	Restoration of FMN survival	–	Serpe et al. (2003)
Axotomy of the facial nerve	Mouse, BALB/c WT, RAG-2 KO, OVA-specific DO11. 10 TCR−/−transgenic+C57BL/6 male and female WT, RAG-2 KO, and MHC II KO	Reconstitution of RAG-2 KO mice with wild-type or OVA-specific CD4+ T cells	–	Wild-type CD4+ T cells restore FMN survival; OVA-specific CD4+ T cells reactive to non-CNS antigen fail to support FMN survival	–	Byram et al. (2004)

(selected studies are summarized in Table 1). The therapeutic outcome of this concept might be the administration of autoreactive T cells or a vaccination strategy with CNS antigens to expand autoreactive T cell clones. Such approaches have been proposed as a potential clinical therapy for a variety of neurodegenerative conditions, including spinal cord injury (Hauben et al., 2001b; Schwartz et al., 1999), Alzheimer's disease (Janus et al., 2000; Morgan et al., 2000; Schenk et al., 1999), glaucoma (Fisher et al., 2001), amyotrophic lateral sclerosis (Angelov et al., 2003) and Parkinson's disease (Benner et al., 2004).

However, the concept of "protective autoimmunity" and corresponding clinical approaches have been severely criticized (Jones et al., 2004, 2002; Popovich and Jones, 2003). Promising results in animal models may not be reproducible in human trials. For example, one potential therapeutic application in the case of Alzheimer's disease involves immunizing patients against the amyloid-β peptide, the major proteinaceous component that characterizes plaques in the brains of patients with this disease. Experimental data in mice (Janus et al., 2000; Morgan et al., 2000; Schenk et al., 1999) led to the development and clinical trials of a vaccine, AN-1792, based on this approach. Unfortunately, several patients in the trial developed CNS inflammation and the vaccine was withdrawn from human trials (Bishop et al., 2002; Check, 2002).

Therefore, further research is necessary to understand the underlying mechanism of protective autoimmunity. Suggested potential mechanisms include the local production of neurotrophins and cytokines by T cells. These are said to cause microglia to buffer toxic mediators such as glutamate, produce growth factors and remove growth inhibitors, for example, by phagocytosis of myelin (Schwartz, 2005).

4. T helper cell subpopulations and CNS injury

One possible explanation for T cell injections and vaccination strategies leading to conflicting results may be that different subtypes of T cells are responsible for distinct T cell effects. Here, we review increasing evidence that a specific T helper cell subpopulation is particularly beneficial in the context of CNS lesions.

4.1. The Th1/Th2 paradigm

T helper cell differentiation and associated effector responses have been intensively studied over the last three decades, initiated by the identification and characterization of cell-mediated versus humoral immunity (Cherwinski et al., 1987; Parish, 1972). In 1986 it was formally shown by Mosmann et al. that mouse CD4+ T cells could be subdivided on the basis of cytokine secretion patterns into two subsets, designated T helper cells type 1 (Th1) and type 2 (Th2) (Mosmann et al., 1986). This conclusion was later extended to human CD4+ T cells (Parronchi et al., 1991; Wierenga et al., 1990) and had an enormous impact on basic and applied immunology (Liew, 2002).

Table 2
Potential correlation of systemic Th1/Th2 balance, EAE resistance and protective autoimmunity in different mouse strains

	Mouse						Rat		
	Balb/c/OLA	C57BL/6	B10.PL	SJL	C3 H/He J	C3H/SW	Fischer	Sprague-Dawley	Lewis
Th status	Th2	Th1	Mixed type	Th1	Th2	Th2	Th2	Mixed type	Th1
EAE resistance	Yes	No	No	No	Yes	Yes	Yes	Yes	No
Protective autoimmunity	+	−	−	−	+	+	+	+	−

After initial activation by immunogenic peptides presented by antigen-presenting cells (APC), naïve Th lymphocytes can differentiate into the phenotypically distinct memory Th1 or Th2 cells.

Th1 cells are characterized by their marker cytokines such as interleukin-2 (IL-2) and interferon-γ (IFN-γ). They activate macrophages and are very effective in controlling infection with intracellular pathogens. In contrast, the marker cytokines of Th2 cells are IL-4, IL-5, and IL-13. These stimulate B cells to produce antibodies and play a major role in eradicating helminths and extracellular parasites. In addition, other CD4+ T cell populations have been described, such as Th 17 T cells that secrete IL-17 and IL-6, as well as regulatory T cell populations that secrete high levels of IL-10 and TGF-β and display the potential to modulate both Th1 and Th2 responses (comprehensive reviews in Biedermann et al., 2004; Shevach, 2002; Weaver et al., 2006; Weiner, 2001).

4.2. Characterization of the Th1/Th2 status

To analyse and compare the Th 1/Th2 status in rodents and humans *in vivo* is more complicated than *in vitro* since several types of tissues and cells have been used to determine it. Some authors compare selected Th1 and Th2 cytokines in an affected tissue (for example in skin homogenates of mice with atopic dermatitis compared to healthy controls). However, it is important to note that these cytokines are not specific to Th1 or Th2 cells. For example, the Th1 cytokines INFγ and tumor necrosis factor-α (TNFα) are expressed by other cell types such as natural killer cells and macrophages, while the Th2 cytokines IL-4, IL-5 and IL-13 are also expressed by mast cells. Other investigators analyze Th1 and Th2 cytokines in the supernatant of stimulated cells isolated from local lymph nodes, spleen, thymus or blood (for example Con A-stimulated pooled splenocytes or blood-derived CD4+ T cells).

To determine whether an experimental test group of animals or humans shows a shift towards a Th1- or Th2-dominated state compared to controls it is useful to calculate a Th1/Th2 ratio. There are several methods: it can be calculated by dividing the number of Th1 cytokine-producing T cells by the number of Th2 cytokine-producing T cells detected for example by FACS analysis. Another method is determining the concentration of selected Th1 and Th2 cytokines in the supernatant of isolated and cultured T cells, for example by ELISA or cytometric bead array. In the first case, a Th2 shift is the result of reduced numbers of Th1 cells or increased numbers of Th2 cells or both. In the second case, a Th2 shift is either the result of decreased Th1 cytokine secretion or increased Th2 cytokine secretion or both.

4.3. EAE susceptibility, protective autoimmunity and the strain-dependent Th1/Th2 status

The systemic Th1/Th2 balance is dependent on age and genetic background (Caruso et al., 1996; Castle et al., 1997; Dayan et al., 2000; Engwerda et al., 1996; Ernst et al., 1993; Frasca et al., 1997; Ginaldi et al., 1999; Kariv et al., 1992; Kubo and Cinader, 1990; Sandmand et al., 2002; Segal et al., 1997; Shearer, 1997). During aging, the Th1/Th2 status passes through three distinct phases (Shearer, 1997). A net Th2 dominance marks the period from birth to young adulthood, followed by a relatively stable Th1/Th2 equilibrium during the reproductive years, except during the period of pregnancy. During the post-reproductive years a net Th2 dominance again emerges (Ginaldi et al., 1999; Sandmand et al., 2002). Analysis of healthy, untreated rodents has revealed important strain differences between the base-line Th1/Th2 status (Table 2) and an age-dependent switch from a Th1 to a Th2 status (Dayan et al., 2000). These strain- and age-dependent differences make comparing different experiments or studies particularly difficult. For example, in healthy, untreated C3H.SW and BALB/c mice the levels of the Th2 cytokines IL-4 and IL-10 increased from undetectable levels in young to significantly high levels in aged mice. The shift to a predominant production of Th2 type cytokines was detectable by 12 months of age, while the Th1 cytokines IL-2 and INFγ decreased with age (Dayan et al., 2000). In contrast, C57BL/6 mice demonstrated hardly any detectable production of IL-4, IL-10, IL-1, and TNFα in all age groups (3–13 months). Only IFNγ levels were high in the young and decreased thereafter (Dayan et al., 2000). In this context, C57BL/6 mice may be considered a Th1-dominated strain, while C3H.SW and BALB/c mice represent Th2-dominated strains. Interestingly, the generalizing characterization of a mouse strain as either Th1 or Th2 suits many strains that exhibit susceptibility for EAE and spontaneous "protective autoimmunity": It has been reported that the ability to exhibit a protective autoimmune T cell response is related to the inherent resistance of a strain to the development of EAE when challenged with myelin-associated antigens (Kipnis et al., 2001, Table 2). Thus, EAE-resistant Balb/c/OLA mice displayed a higher survival rate of retinal ganglion cells (RGC) after optic nerve injury than EAE-susceptible B10.

PL, C57BL/6, SJL/J mice. EAE-resistant C3H/HeJ mice showed more RGC survival than EAE-susceptible C3H/SW mice. Similarly, EAE-resistant Fischer and Sprague-Dawley rats display less RGC death than EAE-susceptible Lewis rats (Kipnis et al., 2001). In other words: more EAE resistance means more protective autoimmunity. More EAE susceptibility (= less EAE resistance) results in less protective autoimmunity. Based on these observations it is tempting to speculate that Th1/Th2 status might play a major role because those strains considered Th1-dominated strains are susceptible (= not resistant) to EAE and display spontaneous "protective autoimmunity" (Table 2). However, the results are less clear in a model of spinal cord injury and consequently there is controversy about a potential relationship between EAE susceptibility and "protective autoimmunity" (Kigerl et al., 2006).

4.4. Are T cell subtypes responsible for neuroprotective and pro-regenerative effects?

Surprisingly, only a few studies have further characterized those T cell subtypes exerting beneficial effects in the context of CNS injury. For example, in a model of murine entorhinal-hippocampal brain slice cultures it could be demonstrated *in vitro* that Th2 cells prevented or even reversed Th1-induced upregulation of the inflammatory marker ICAM-1 on microglial cells (Gimsa et al., 2001). Furthermore, in a similar model, both Th1 and Th2 cells were able to support neuronal survival provided there was no cell–cell contact with the slices. Th2 cells displayed a significantly higher protective effect compared to Th1 cells (Fig. 2; Wolf et al., 2002). An *in vivo* study by the Schwartz group demonstrates that injections of autoimmune Th1 cells (characterized by IFNγ and IL-10 secretion patterns) lead to neuroprotection compared to PBS injection (Kipnis et al., 2002). Unfortunately, no Th2 cells were used in this study. These data suggest that both Th1 and, to a greater extent, Th2 cells may support neuroprotection and regeneration.

5. Potent inducers of a Th2 shift promote neuroprotection and neuroregeneration

Several studies have shown that potent inducers of a Th2 shift such as Th2-inducing adjuvants, glatiramer acetate or members of the statin family improve outcome after CNS injury.

5.1. Th2-inducing adjuvants (IFA, Alum)

The selection of adjuvant used for immunization during vaccine strategies determines either a predominantly Th1- or a Th2-dominated status. Complete Freund's adjuvant (CFA) supports a Th1 status, while in complete Freund's adjuvant (IFA) promotes a Th2 status (Shibaki and Katz, 2002). Aluminum hydroxide (Alum) is another adjuvant that supports a dominant Th2 status in mice (Comoy et al., 1997; Cox and Coulter, 1997; Grun and Maurer, 1989; Pollock et al., 2003).

Importantly, in the context of vaccination strategies for neuroprotection and regeneration, it should be taken into account that the use of a specific adjuvant might lead to brain inflammation (CFA) or allergic responses (Alum) and may have different effects in mice than in humans (Francis and Durham, 2004). CFA contains inactivated mycobacteria, which are responsible for inducing the Th1 shift of the immune response, while IFA does not contain mycobacteria.

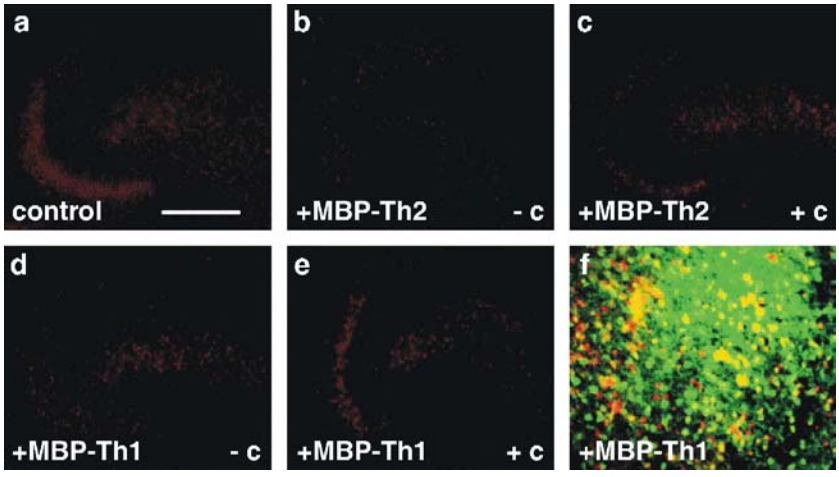

Fig. 2. Representative photomicrographs show neuronal damage in slices 24 h after treatment with MBP-specific Th1 and Th2 cells. The nuclei of dead cells are stained with propidiumiodide and appear in a red fluorescence. (a) Control slices without T cells showed the most neuronal damage. (b) MBP-specific Th2 cells without contact (−c) showed the highest protective potential. (c) When MBP-specific Th2 cells were in contact (+c) with the brain tissue, their protective potential was significantly decreased. (d) MBP-specific Th1 cells without contact to the slices were also neuroprotective. (e) MBP-specific Th1 cells in contact with neuronal tissue were not protective. (f) Double staining of neurons with neurofilament and propidiumiodide shows that the nuclei of dead cells belong to neurons in this region of the hippocampus. This micrograph shows the survival rate after treatment with MBP-specific Th1 cells without contact to the slices. Scale bar in picture (a) is representative for all photomicrographs: 0.5 μm. (Reproduced with written permission from Wolf et al., 2002).

It is tempting to use IFA or Alum (instead of CFA) for the immunization of mammals with encephalitogenic proteins (Hauben et al., 2001a) because such a protocol appears to prevent EAE in immunized animals (Killen and Swanborg, 1982; Namikawa et al., 1982).

Using CFA and IFA as adjuvants for immunization with encephalitogenic proteins significantly increased neuroprotection after optic nerve crush, with slightly higher neuronal survival rates in the IFA/Th2 group (Kipnis et al., 2002). In a model of aseptic cerebral injury, CFA and IFA have been compared as adjuvants for immunization with MOG and OVA (Hofstetter et al., 2003). Independent of adjuvant or antigen, immunization had a clear beneficial effect. Unfortunately, the "healing index" used to analyze the effects is only roughly defined (cellular infiltration, necrosis, vascularity) and neuronal damage has not been investigated (Hofstetter et al., 2003). In two excellent studies on long-distance axon regeneration after spinal cord injury, IFA and Alum were used as adjuvants (Huang et al., 1999; Sicotte et al., 2003). Alum was the most effective in promoting long-distance axon regeneration — without inducing EAE (Sicotte et al., 2003).

5.1.1. Glatiramer acetate

Glatiramer acetate (GA, Copolymer-1, Copaxone) is a potent inducer of a Th2 switch (Dhib-Jalbut et al., 2003; Duda et al., 2000; Farina et al., 2001; Gran et al., 2000; Neuhaus et al., 2000). It is a synthetic basic random copolymer of L-glutamic acid, L-lysine, L-alanine, and L-tyrosine and was originally studied in an attempt to simulate the activity of myelin basic protein in inducing EAE. However, it was then found to suppress EAE in various species (reviewed in Hohlfeld and Dalakas, 2003).

There is accumulating evidence that GA or GA-specific T cells exert neuroprotective effects after mechanical lesion of the CNS (Kipnis et al., 2000; Schori et al., 2001) or PNS (Angelov et al., 2003). Active immunization with GA administered in adjuvants as well as adoptive transfer of T cells reactive to GA can inhibit the progression of secondary degeneration after crush injury to the rat optic nerve (Kipnis et al., 2000). Immunization with GA emulsified in CFA protects retinal ganglion cells against death from glutamate cytotoxicity and ocular hypertension (Schori et al., 2001). In acute neuronal degeneration after facial nerve axotomy, the number of surviving motor neurons was almost two times higher in GA-vaccinated mice than in non-vaccinated mice and mice injected with PBS emulsified in complete Freund's adjuvant (Angelov et al., 2003).

5.1.2. Statins

Statins are inhibitors of the HMG-CoA reductase and several members of the statin family (including cerivastatin, simvastatin, lovastatin, and atorvastatin) have been shown to be potent inducers of a Th2 switch (Aktas et al., 2003; Arora et al., 2006; Hakamada-Taguchi et al., 2003; Youssef et al., 2002). They exert neuroprotective effects in several CNS diseases including Alzheimer's disease, MS and stroke (review in Stepien et al., 2005). Atorvastatin administration results in beneficial effects on neurological outcome, synaptogenesis, angiogenesis and neuronal survival have been shown after traumatic brain injury (Lu et al., 2004a,b,c; Qu et al., 2005) and reduces the inflammatory response after SCI (Pannu et al., 2005).

These studies support the concept that potent Th2 inducers such as GA and statins promote neuroprotection and regeneration.

6. CNS injury results in a systemic Th2 shift

A potential beneficial role of Th2 cells in neuroregeneration is illustrated by the fact that CNS injury is associated with a systemic Th2 shift. CNS injury induces a substantial modulation of the immune system, which leads to secondary immunodeficiency (CNS injury-induced immunodepression [CIDS]) (Meisel et al., 2005). CIDS results in increased susceptibility to infection, the leading cause of morbidity and mortality in patients with acute CNS injury. Interestingly, this immunodepression is characterized by a catecholamine-driven systemic Th2 shift in mice (Prass et al., 2003) and humans (Dirnagl, manuscript in preparation), which leads to impaired cellular immune responses and decreased IFNγ production by blood lymphocytes, while humoral immune responses were less affected (comprehensively reviewed in Meisel et al., 2005). In a mouse model of stroke, the adoptive transfer of IFNγ-producing lymphocytes from healthy littermates or treatment with recombinant IFNγ greatly diminished bacterial burden demonstrating that a Th2 switch plays a causal role in CIDS persistence and consecutive infections (Prass et al., 2003). Thus, it is feasible that CIDS may not be limited to counterbalancing excessive inflammation in the brain. Since CNS injury is associated with the activation of autoreactive T cells (see above), the associated Th2 shift may suppress cellular immune responses that induce autoimmune brain inflammation (similar to the immunization models using Th2-inducing adjuvants).

7. Is a Th2 status beneficial for the injured CNS?

Importantly, in the two studies of the David lab summarized above, IFA or Alum as adjuvants potently promoted axon regeneration but prevented the development of EAE, in contrast to CFA (Huang et al., 1999; Sicotte et al., 2003). Thus, it is tempting to speculate that a downregulation of Th1 responses and a shift towards Th2 responses after CNS damage is a *physiological wound healing reaction* following CNS injury in order to prevent the development of autoimmune diseases such as EAE and MS.

To make things even more complicated, there is considerable debate about the Th1/Th2 paradigm in EAE and MS. Recent data suggest that IL-17 and IL-23 (rather than the classical Th1 cytokines IL-12 and IFNγ) are key cytokines in the initiation and persistence of EAE (review in

McGeachy and Anderton, 2005). Thus, potential beneficial effects of a Th2 switch may in fact represent altered functions of Th17-producing T helper cells and IL-23-producing macrophages and dendritic cells.

Briefly, after CNS injury a systemic Th2 shift may be important in downregulating Th1- or Th17-driven cellular immune responses in order to prevent autoimmune diseases such as EAE or MS. The price for the systemic Th2 shift is increased susceptibility to infection. An additional Th2 shift may further increase neuroprotection and regeneration.

8. Conclusions

There are several lines of evidence that a Th2 switch is beneficial for the injured CNS:

1. Th2 cells support neuronal survival better than Th1 cells *in vitro* (Wolf et al., 2002).
2. Th2 cells suppress Th1-induced inflammatory signals in brain slices *in vitro* (Gimsa et al., 2001).
3. CNS injury induces systemic immunosuppression characterized by a systemic Th2 switch, which impairs cellular immune responses (Meisel et al., 2005).
4. In vaccination models for the treatment of CNS injury, Th2-inducing adjuvants such as IFA and Alum promote axon regeneration better than the Th1-inducing adjuvant CFA (Huang et al., 1999; Sicotte et al., 2003).
5. In vaccination models for the treatment of CNS damage, Th2-inducing adjuvants prevent the development of autoimmune diseases such as EAE (Huang et al., 1999; Sicotte et al., 2003).
6. Potent inducers of a systemic Th2 switch such as GA and statins support neuroprotection and/or regeneration (Lu et al., 2004a; Pannu et al., 2005).

Hence, there is good evidence that both Th1 cells and Th2 cells promote neuroprotection and regeneration and that vaccine strategies may exert beneficial effects during CNS wound healing in several (but not all) models investigated. There is increasing evidence *in vitro* and *in vivo* that Th2 cells or a systemic Th2 shift may *further* increase neuroprotection and axon regeneration after CNS injury — but most importantly, a Th2 shift seems to prevent EAE after immunization with CNS antigens.

9. Future perspectives

A systematic investigation of the influence of Th1/Th2 status on neuroprotection and axon regeneration has not yet been carried out. Therefore, it would be advantageous to analyze Th1- and Th2-dominated experimental models for diverging outcomes in both lesion development after CNS lesion and the results of therapeutic interventions such as T cell injections and vaccine strategies. Within the context of vaccination strategies for neuroprotection and regeneration, it should be taken into account that the use of either Th1- or Th2-inducing adjuvants might lead to brain inflammation (CFA) or allergic responses (Alum) and may have different effects in rodents than humans (Francis and Durham, 2004). It is important to analyze a potential increase of CIDS-induced susceptibility to infection as a side effect of a therapeutic Th2 switch. However, investigating whether Th2-inducing adjuvants reduce autoimmune side effects in vaccination models for the treatment of CNS injury holds particular promise. Finally, more studies are needed to investigate whether a therapeutic Th2 switch further promotes neuronal survival and axonal regeneration after CNS damage and what potential mechanisms may be involved.

In summary, there is a lot of experimental evidence that T helper cells play a beneficial role in the treatment of CNS injury, however, there are still many questions requiring answers regarding the potential side-effects of T cell injections and vaccine strategies.

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