45
Protective and Regenerative Autoimmunity in CNS Injury

Jonathan Kipnis and Michal Schwartz

Keywords Adaptive immunity; Autoimmune; CD4CD25; Lymphocytes; Microglia; Neurotrophins; T cells; Spinal cord injury; Vaccination; Wallerian degeneration

45.1. Introduction

Neurodegenerative diseases are generally considered to be non-inflammatory, unlike autoimmune diseases such as multiple sclerosis, which are neurodegenerative diseases that are inflammatory in nature (Trapp et al., 1999a, b; Hohlfeld and Wiendl, 2001; Groom et al., 2003). Nevertheless, most neurodegenerative diseases are accompanied by a local inflammatory response, widely assumed to be unfavorable for CNS recovery (Kurosinski and Gotz, 2002; Jellinger, 2003; Popovich and Jones, 2003). Moreover, the progressive degeneration seen in such diseases is often mediated by compounds and processes that are secondary to the primary risk, e.g., misfolding and aggregation of self-proteins (Shastry, 2003). These primary and secondary risk factors represent a continuous threat to any viable neurons embedded in a chronically diseased tissue; they induce abnormalities in cells in their vicinity, thereby contributing to the chaos rather than helping to resolve it.

Another feature common to most of the chronic neurodegenerative conditions is age dependence. The incidence of Alzheimer’s and Parkinson’s disease, glaucoma, and many others increases significantly with age (Ossowska, 1993; Mattson, 2003). As will be discussed below the age factor might not be related only to the aging of the brain but also to age-related changes in the immune system. Often the removal of the primary risk factor does not stop disease progression, and the neurodegeneration continues (Weinreb and Levin, 1999; Schwartz and Cohen, 2000). In Parkinson’s disease, for example, despite dopamine (L-dopa) treatment as a replacement therapy the dopaminergic neurons continue to die (Hirsch, 1999; Montastruc et al., 1999). In glaucoma, reduction of intraocular pressure often does not stop disease progression (Schwartz et al., 1996). This progressive degeneration, which continues despite removal of the presumed primary risk factor(s), has been linked to what has been recognized as secondary degeneration. Emerging risk factors have been attributed to this phenomenon, including inflammation-associated factors. This has been studied intensively in recent years with a major focus on the balance between the benefit and the risk of uncontrolled immune activity, which exceeds the ability of the CNS to tolerate it.

45.1.1. Inflammation—A Local Response in Acute CNS Insults: Is It Always Bad?

The function of inflammation after any acute or chronic insult to the CNS has long been a matter of debate. Concepts such as the immune-privileged status of the CNS, as well as observations such as the presence of immune cells in the diseased CNS, gave rise to the prevailing belief that immune activity in the CNS is detrimental (Lotan et al., 1994). Many researchers consider inflammation to be an important mediator of secondary damage (Dusart and Schwab, 1994; Carlson et al., 1998; Fitch et al., 1999; Popovich et al., 1999; Mautes et al., 2000). Fitch et al. (1999) demonstrated that inflammatory processes alone can initiate a cascade of secondary tissue damage, progressive cavitation, and glial scarring in the CNS, and they suggested that specific molecules which promote inflammation might play a role in initiating secondary neuropathology (Fitch et al., 1999). This is apparently in line with the finding that removal of macrophages after SCI might improve the functional outcome (Popovich et al., 1999), and with the reported observations that the anti-inflammatory compound methylprednisolone promotes recovery in spinally injured rats (Constantini and Young, 1994). Other studies, however, indicate that local immune activity may have a beneficial effect on the traumatized spinal cord through clearance of cell debris and secretion of neurotrophic factors and cytokines. Macrophages and microglia promote axonal regeneration by clearing the site of injury (David et al., 1990; Perry et al., 1992; Madsen et al., 1998; Rapalino et al., 1998; Fitch et al., 1999; Fischer et al., 2004), and T cells mediate processes of maintenance.
and repair and promote functional recovery from CNS trauma (Moalem et al., 1999b; Hauben et al., 2000a, b; Schwartz et al., 2001; Ling et al., 2006). Guth and his colleagues suggested a therapeutic combination of anti-inflammatory drugs such as allapenol (to inhibit injury-induced xanthine oxidase), indomethacin (to inhibit constitutive and inducible cyclooxygenase), and bacterial lipopolysaccharide (LPS) to stimulate macrophage activity. The short-term effect of the suggested therapy, however, while including both anti- and pro-inflammatory treatment, appears to be different from its long-run effect. Along the same lines, it was suggested that animals with limited ability to undergo Wallerian degeneration might suffer from limited wound healing and regeneration (Zhang and Guth, 1997). These and other studies led researchers to acknowledge that some aspects of inflammation have negative effects on recovery, whereas others are beneficial and even essential. (Bethea et al., 1999; Mason et al., 2001)As more and more pieces are added to the puzzle of post-traumatic CNS inflammation it becomes increasingly evident that to describe the effect of inflammation on the injured nerve as “good” or “bad” is an oversimplification, as it reflects the common view of inflammation as a single (and deleterious) process rather than as a series of local immune response that is primarily being recruited to cope with threat. Its ultimate benefit, lack of benefit, or even destruction is a reflection of regulation and timing and the ability of the tissue to cope with the harmful effects of the factors produced by the immune response. Conflicting interpretations of inflammation might thus reflect, the experimental injury model employed, the severity of the injury, time elapsed following injury, the markers used to identify locally activated immune cells, the species, and the strain. In addition, the choice immune-based manipulation used to demonstrate adverse effect is also critically affecting the outcome. Immune response being the physiological mechanism by which the body copes with damage, is essential for recovery, but is more constructive when suitably regulated.

45.1.2. Role of Innate Immunity in CNS Repair

Healing of tissue in response to injury involves the synchronized operation of numerous factors and processes, some of them operating in concert and others in sequence. It is essential that the set of processes occurring in the injured axons be synchronized with the set occurring in the cells surrounding the injured axons, such that the axonal environment acquires growth-supportive properties and the axons acquire growth activity. It seems plausible that the two sets of processes operate, but that the proper synchronization is lacking. Acquisition of growth supportive properties by the cellular milieu of the injured axons basically means achievement of a balanced environment for regrowth and cell renewal (Butovsky et al., 2001, 2005a, b, 2006; Ziv et al., 2006b). This synchrony might be achieved by an appropriate postinjury immune response that is compatible with the CNS ability to tolerate.

45.1.3. Lessons from Peripheral Nervous System—Wallerian Degeneration: Is It Needed for Repair?

Central nervous system (CNS) response to injury has long been viewed as though the CNS is a unique tissue whose behavior after injury is governed by different rules than those underlying the response of other types of injured tissues, including the peripheral nervous system (PNS). Accordingly, although failure of the CNS to regenerate has been intensively studied over the years, the usual approach has been to regard the CNS as atypical and therefore to study it as an entity distinct from any other tissue. On the assumption that CNS healing does not necessarily differ in principle from the healing of any other tissue, attempts to uncover the reasons for regeneration failure have begun to focus on comparisons, both phylogenetic and intraspecies, between regenerative and nonregenerative nervous systems. The loss of function following CNS injury has been attributed not only to the failure of regeneration but also to the secondary damage which is a mechanism whereby the spread of damage from directly injured neurons to neurons that escaped the primary lesion (Robertson et al., 2000; Schwartz and Yoles, 2000; Taoka et al., 2000; Schwartz, 2001a; Vajda, 2002; Wu, 2005). In the past two decades it has become clear that failure of CNS regeneration might be partly due to the inability of the cellular elements surrounding the injured axons to create a balanced environment capable of permitting and supporting regrowth (Caroni et al., 1988; Schwab and Bartholdi, 1996; Rapalino et al., 1998; Schwab, 2002). It was shown initially that transected CNS axons, which fail to regenerate in their own degenerative environment, were shown to be capable of growing into transplanted peripheral nerve bridges (Aguayo et al., 1984; So and Aguayo, 1985; Aguayo et al., 1987; Vidal-Sanz et al., 1987). Among the elements that were shown to be hostile to regrowth in adult CNS nerves, and are absent during development, are myelin-associated inhibitors (Tang et al., 2001; Domeniconi et al., 2002; Kim et al., 2004; Schwab et al., 2005). These inhibitors, over the years, have been fully characterized and are known as the NoGo family (Tatagiba et al., 1997; Merkler et al., 2003). NoGo has three major spliced isoforms (termed Nogo-A, -B and -C) that share similar domain structures. Proteins are highly expressed in oligodendrocytes, the longest of these, Nogo-A, has a large N-terminus followed by two putative membrane-spanning domains and a short C-terminal segment. The 66-amino acid segment (known as the Nogo-66 domain) between the two transmembrane domains is extracellular. A neuronal Nogo-66 receptor (NgR) that interacts in trans with the oligodendrocyte Nogo-66 domain has also been identified. Exogenous NgR expression in neurons that are otherwise not susceptible to Nogo-mediated growth inhibition confers inhibitory susceptibility, indicating that NgR could functionally transduce at least part of the inhibitory signal presented by Nogo. It was suggested that for regeneration to occur, these inhibitors must be either masked or eliminated. In the nervous systems of different species, the levels of such
inhibitors and/or the extent of their postinjury removal might correlate with regenerative capacity. For example, in mammalian CNS there is a high level of such inhibitors, whereas in lower vertebrates their level is lower and their removal seems to be more efficient (Lang et al., 1995; Hirsch and Bahr, 1999). Subsequent studies have identified additional myelin-associated proteins as inhibitors of regrowth, such as MAG (Tang et al., 2001; Domeniconi et al., 2002). In addition, it was suggested that the rate of myelin clearance from the CNS is significantly lower than from the PNS (Hofer et al., 1981; Pellegrino et al., 1986; Stoll et al., 1989). Another set of studies have suggested that astrocytes, thought to be involved in scar formation, are needed for growth support, and their failure to support axonal growth after injury to the mammalian CNS might be related to their cellular properties and the nature of their extracellular milieu, such as production of chondroitin sulphate proteoglycan (Jones et al., 2002; Moon et al., 2002; Jones et al., 2003; Sandvig et al., 2004, Hofer et al., 1981; Pellegrino et al., 1986; Stoll et al., 1989). Yet, recent studies from our laboratory have suggested that although the overall production of the CSPG might be inhibitory of regrowth, complete inhibition of its production worsened the overall recovery, its early production is needed but should be limited (Rolls et al. unpublished observation). Thus, it appeared that timing and intensity of the glial scar and the local immune response are critically determining the recovery, as is discussed below. These and other related findings suggest that the postinjury behavior of cells surrounding the injured axons might determine the regenerative capacity of the axons. (Dolenc, 1984; Ogawa et al., 1985; Dolenc, 1986; Angaut-Petit and Faille, 1987; Kalichman and Myers, 1987; Gu and Ma, 1991; Berkenbosch, 1992; Sirron et al., 1994; Bregman et al., 1995; Tidball, 1995; diZerega, 1997; Hirsch and Bahr, 1999; Best and Hunter, 2000; Fry, 2001; Kalla et al., 2001; Lakatos and Franklin, 2002; Cui et al., 2004; Fenrich and Gordon, 2004; Ferraro et al., 2004).

45.1.4. Macrophages/Microglia in CNS Repair

Wound healing is a complex, multistep process involving reciprocal interactions between immune cells from the circulation and resident cells of the tissue, with the participation of extracellular matrix proteins and an array of bioactive molecules with multiple actions. In most parts of the body, tissue injury triggers immediate infiltration of circulating immune cells into the damaged area. Twenty four hours after injury, infiltrating monocytes represent the majority of leukocytes at the site of injury. Migration and adhesion of monocytes are controlled by secreted factors, whose autocrine or paracrine activity promotes recruitment of those immune cells (Lazarov-Spiegler et al., 1999; Kalla et al., 2001). On reaching the tissue, the monocytes are locally activated and became ‘alternatively’ activated macrophages. The macrophages play a central role in wound healing by clearing debris from the injury site (Stoll et al., 1989; Schwab et al., 2001). Macrophages secrete cytokines, growth factors and enzymes into the wound site, and participate in a profusion of autocrine and paracrine reactions with invading immune cells and resident tissue cells. When macrophages are eliminated by local injection of anti-leukocyte serum, or when monocyte production is prevented by injection of glucocorticoids, wound healing proceeds very slowly (Shirafuji et al., 2001, 2003).

In regenerating neural tissues, macrophages appear to be involved in both degeneration and regeneration. In the PNS, there is immediate Wallerian (i.e. anterograde) degeneration of the distal stump, involving the breakdown of axons and the fragmentation of Schwann cell cytoplasm. The invading monocytes play a major role in this process by clearing myelin debris and degenerating fibers, and by facilitating Schwann cell proliferation. In addition, they provide substances that participate in the healing process; for example, macrophage-derived apolipoproteins are expressed at the injury site and probably participate in membrane rebuilding. Nerve growth factor (NGF) synthesis seems to be regulated by the stimulated macrophages. Interleukin 1 and tumor necrosis factor a, secreted by macrophages, probably induce NGF transcription in Schwann cells (Stoll et al., 1989; Frisen et al., 1994; Schwab et al., 2000; Rotshenker, 2003; Stoll et al., 2004). Comparative in vivo and in vitro studies of the mammalian CNS and PNS have pointed to the part played by macrophages in the axonal response to injury, as well as to the link between macrophage activity and the success or failure of regeneration (Griffin et al., 1992; Giulian et al., 1995; Leskovar et al., 2000). In the CNS, in contrast to the PNS, macrophage infiltration following axonal injury is not only delayed but also restricted to the lesion site, rather than being dispersed along the part of the nerve through which the newly growing axons should elongate (Lazarov-Spiegler et al., 1998; Stichel et al., 1999; Wang and Feuerstein, 2000; Dihne et al., 2001; Schwartz, 2001a; Sekiya et al., 2001; Franzen et al., 2004). Furthermore, unlike in regenerative tissues, where invading blood-borne monocytes are the prominent inflammatory cells, in the CNS the resident microglia are considered to be the major mononuclear phagocytic participants at the site of injury. Following injury, activated microglia and infiltrating blood-borne macrophages are immunohistochemically indistinguishable. As the quiescent resident microglia in the intact CNS are thought to be in a down regulated form, it is possible that, following injury, the extent and the nature of their activation, in terms of acquiring healing-supportive activities, is limited compared with that of other tissue-resident macrophages. In vitro studies have shown, for example, that cytokines and growth factors associated with inflammation and wound healing have a significant effect on scar formation and/ or dissolution. These factors appear to affect protease production, production of cross-linking enzymes (such as transglutaminase) and production of extracellular matrix proteins, all known significantly to affect the ability of astrocytes to support growth. Other studies have suggested that macrophage-derived factors have a cytotoxic effect on oligodendrocytes (Griot et al., 1989; Griot-Wenk et al., 1991; Zajicek et al., 1992). The above observations, together with other studies point to a link

45. Protective and Regenerative Autoimmunity in CNS Injury
between failure of CNS regeneration and the limitations in rate, activity and distribution of the immune cells in response to CNS injury.

45.1.4.1. The Rationale for Macrophage Therapy and Its Preclinical Characteristics

Experimental results over the last decade suggest that macrophages and brain microglia are multitalented cells that are capable of expressing different functional programs in response to distinct micro-environmental signals. Microbial products and cytokines profoundly affect the differentiation of monocytes towards two phenotypic extremes. Microbial products are associated with the ‘classical’ activation of monocytes/microglia. The ‘classically’ activated macrophages are potent effector cells that kill microorganisms and tumor cells. This ‘killer instinct’ have been long viewed as the only the main function of microglia. In contrast, “alternatively” activated macrophages tune inflammatory response and adaptive immunity, scavenge debris, and promote angiogenesis, tissue remodeling and repair (Summers et al., 1995; Klusman and Schwab, 1997; Mantovani et al., 2002; Bomstein et al., 2003; Gordon et al., 2003; Hauben et al., 2003; Mosser, 2003). Similarly, microglia activated by adaptive immunity are microglia that can present antigens, produce growth factors, buffer glutamate and support cell renewal (Shaked et al., 2004; Butovsky et al., 2005a, b; Shaked et al., 2005; Butovsky et al., 2006).

Initial experiments in animals with complete spinal cord transection demonstrated that local application of macrophages that have been co-incubated with sciatic nerve promoted motor recovery (Lazarov-Spiegler et al., 1996; Rapalino et al., 1998). Subsequently, the experiments were repeated in a model of severe spinal cord contusion. In those experiments blood-borne monocytes were activated by co-incubation with autogeneous skin (Bomstein et al., 2003). The results revealed that this was equally effective for recovery from spinal cord injury. In these and subsequent experiments, the macrophages were characterized phenotypically, and parameters such as site of injection, dosing and therapeutic window were studied. Those macrophages were found to have a dendritic-like phenotype (Bomstein et al., 2003) and expressed features that are reminiscent of ‘alternative activation’: production of low levels of proinflammatory cytokines, and production of growth factors and metaloproteases. In subsequent studies aiming at finding the optimal time for intervention with local implantation of macrophages it became clear that as in any other tissue, repair and restoration are not only dependent on location and context, but also timing. Table 45.1 describes time windows representing a different physiological stages following SCI.

Taken together, the results summarized above as well as additional studies, it is suggestive that timely local innate immune cells with “alternative activity,” reminiscence of dendritic-like, are required for CNS ability to cope with injurious conditions, and that they are needed at the sub-acute phase following injuries.

45.2. Adaptive Immunity Is Needed to Control Local Innate Response in the CNS

45.2.1. Is Self and Non-Self Discrimination Needed?

“Survival of the fittest” summarizes the essence of Darwinian evolutionary theory. In line with this theory and the pioneering theory of Metchnikoff in the 1890s (Dubos, 1955; Vaughan, 1965), followed by the “clonal expansion” theory of Burnet in the 1950s (Miller, 1994; Silverstein and Rose, 1997; Martini and Burgio, 1999), it was believed that discrimination of self from non-self, thymic education of T cells, and deletion of autoimmune T cells in the thymus are the central features of immunology. Self-tolerance, defined as a state of non-responsiveness to self, was therefore viewed as the optimal condition, and was assumed to enable the fittest to survive (Viret et al., 1999). Studies carried out in rodent models of central nervous system (CNS) insults have suggested that autoimmunity is the body’s defense mechanism against any threat to CNS tissue (Schwartz and Cohen, 2000; Schwartz et al., 1999) and that only when the autoimmune response is poorly controlled will an autoimmune disease result. According to these observations and others, it emerged that defining tolerance to self in terms of non-responsiveness is incompatible with the theory of the survival of the fittest. A more appropriate definition of tolerance to self would

<table>
<thead>
<tr>
<th>Days post spinal cord injury</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3–4 days</td>
<td>A period reflecting the decline of primary infiltration of neutrophils participating in inflammation, and high incidence of apoptotic cells.</td>
<td>(Popovich et al., 1997; Leskovar et al., 2000)</td>
</tr>
<tr>
<td>7–10 days</td>
<td>A period of maximum accumulation of activated microglia/macrophages, T cells, and progenitor glial cells.</td>
<td>(Leskovar et al., 2000; McTigue et al., 2001)</td>
</tr>
<tr>
<td>14 days</td>
<td>The numbers of ED1 positive cells and T cells are still very high. At the same time different cytokines and chemokines in the injured tissue decrease or disappear.</td>
<td>(Lee et al., 2000; Leskovar et al., 2000; McTigue et al., 2001)</td>
</tr>
<tr>
<td>21 days</td>
<td>Many of the injury-induced biochemical and cellular activities in the spinal cord have peaked and begun to return to normal levels.</td>
<td>(Leskovar et al., 2000; McTigue et al., 2001)</td>
</tr>
</tbody>
</table>
be the ability to tolerate an anti-self response without developing an autoimmune disease (Schwartz and Kipnis, 2002). Just as the immune system fights off external pathogens, the autoimmune system fights off threats originating within the body itself (such as cancer, neurodegenerative conditions, tissue injuries), and also serves as a complementary defense mechanism against damage caused by external pathogens. Naturally occurring regulatory T cells (CD4+CD25+) serve as a physiological safety valve that can be modulated to maintain the fine balance between need and risk (Kipnis et al., 2002; Schwartz and Kipnis, 2002; Kipnis et al., 2004a).

In the early 1990s it became evident that there is little difference, between the T-cell repertoires of healthy individuals and of patients suffering from autoimmune diseases (Lohse et al., 1996). At around the same time it was suggested that the sole function of a group of suppressor T cells newly identified as CD4+CD25+ was to inhibit the anti-self aggression of any autoimmune T cells that (presumably owing to an evolutionary mistake) had left the thymus and taken up residence in the periphery (Shevach, 2000; Shevach et al., 2001). It is contrary to Darwinian theory, however, to propose that two cell populations exist in the same organism for the sole purpose of inhibiting each other’s activity. Survival of the fittest implies that unwanted features, especially if harmful, will disappear, while beneficial features will be transferred to future generations (Paul, 1988; Herman, 1997; Elliott, 2003). Thus, since all humans possess a similar repertoire of autoimmune cells (Finn et al., 1996; Ria et al., 2001), Darwinian theory would presuppose that these cells have a physiological function.

Theoretically, complete elimination of autoimmune T cells would be the best way to prevent autoimmune disease development, whereas uninhibited autoimmunity would be the best way to counteract neurodegenerative disorders and cancer. A Darwinian resolution of these opposing immunological scenarios might have led, as a compromise between risk and benefit, to the concomitant presence of autoimmune T cells and the regulatory T cells that normally suppress them (Schwartz and Kipnis, 2002). Based on the accumulated information describing the role of autoimmunity in the devastating conditions of cancer (Shimizu et al., 1999; Sakaguchi et al., 2001) and neurodegeneration (Kipnis et al., 2002), it seems unlikely that even the most ardent disciple of Burnet would suggest that complete deletion of autoimmunity favors survival of the fittest. Hence a new theory based on solid data suggests that CD4+CD25+ regulatory T cells do not exist in a permanently suppressive state that keeps autoimmune T cells unresponsive to self-antigens, but are amenable to modulation by physiological signals that weaken or strengthen their suppressive activity according to need.

45.2.2. Autoimmune T Cells Protect Neurons from Degeneration

Autoimmunity has long been viewed as a destructive process. However, a strong body of evidence provides a new view whereby autoimmunity is the body’s endogenous response to CNS injury, and that its purpose is beneficial. This notion was based on the observation that in rodents, passive transfer of encephalitogenic (disease-inducing) T cells reactive to myelin basic protein (MBP) reduces postinjury neuronal losses.

During the last two decades it has become increasingly clear that different degenerative diseases of the CNS share a number of primary and secondary features (Evert et al., 2000; Rehman, 2000; Hur et al., 2002; Carri et al., 2003). In many such diseases the local microglial response is often viewed as an unwelcome contributor to the disease pathology (Aschner et al., 2002; Koutsilieri et al., 2002; Liu et al., 2002). Recent data suggest, however, that such a view is an oversimplification, and that a well-controlled glial response is beneficial in protecting the affected tissues (Banati et al., 1994; Aschner et al., 2002), whereas malfunctioning glia contribute to the ongoing neurodegenerative process (Teismann et al., 2003). This spread of neuronal damage is caused, at least in part, by compounds which, though normally essential for the survival and function of neurons, become toxic when their physiological concentrations are exceeded. Among the injury-related mechanisms that might underlie the post-traumatic spread of damage are biochemical and metabolic changes in oxygen and glucose utilization, energy state, lipid-dependent enzymes, free radicals, eicosanoids, tissue ions, biogenic amines, endogenous opioids, and excitatory amino acids. These changes cause alterations in cellular homeostasis, excitotoxicity, local production of agents harmful to nerve cells, and a loss of trophic support from targets, all of which result in secondary neuronal loss.

Immune responses in the CNS are relatively restricted, resulting in the status of the CNS as an immune-privileged site (Streilein, 1995). The unique nature of the communication between the CNS and the immune system can be observed, for example, in the dialog between the CNS and T cells. Under normal conditions activated T cells can cross the blood–brain barrier and enter the CNS parenchyma. However, only T cells capable of reacting with CNS antigens seem to persist (Hickey et al., 1991). Comparative studies of the T-cell response at sites of axotomy in the CNS and the peripheral nervous system (PNS), using T-cell immunocytochemistry, revealed a significantly greater accumulation of endogenous T cells found in injured PNS axons than in injured CNS axons (Moalem et al., 1999a). Moreover, in cases of inflammation, the CNS showed a marked potential for elimination of T cells via apoptosis, whereas such elimination was less effective in the PNS, and was almost absent in other tissues such as muscle and skin (Gold et al., 1997).

In 1999, it was demonstrated that autoimmune T cells directed against myelin basic protein can protect neurons against degeneration after CNS injury (Moalem et al., 1999b). To verify that this finding was not merely the result of an experimental manipulation but rather a beneficial physiological response to CNS injury, neuronal degeneration after identical injuries was compared in normal mice and mice devoid of T cells (Kipnis et al., 2001; Yoles et al., 2001). Significantly
more degeneration was observed in the nude mice than in the wild type, suggesting that neuroprotection is a physiological, T cell-dependent process (Yoles et al., 2001). To confirm the autoimmune character of this beneficial physiological response, animals were tolerized to myelin antigens at birth. The tolerized animals showed significantly fewer surviving neurons after injury than their matched controls that had been immunized neonatally with an irrelevant (non-myelin) protein (Kipnis et al., 2002).

It had been widely accepted that autoimmune T cells in the periphery are normally kept in a state of tolerance by the suppressive activity of naturally occurring regulatory CD4+CD25+ T cells (Treg cells) (Thornton and Shevach, 2000), and that elimination or depletion of Treg cells might therefore cause development of an autoimmune disease in susceptible animals (McHugh et al., 2002).

Verification of a new perception of autoimmunity is seen in the experiments in which mice were depleted of Treg cells assuming that such a manipulation would increase the ability to fight off neurodegenerative conditions (Kipnis et al., 2002; Kipnis et al., 2004a). Regardless of the mouse’s inherent susceptibility to autoimmune disease, Treg cell depletion resulted in increased neuronal survival (Kipnis et al., 2004b). These and other observations led to propose that the ability to harness a T cell-dependent protective mechanism is controlled by Treg cells, and that the constitutive presence in healthy individuals of both autoimmune T cells and regulatory T cells represents an evolutionary solution to the need for autoimmune T cells for maintenance and repair, with Treg cells acting as a safeguard against the risk of autoimmune disease (Schwartz and Kipnis, 2002). According to these and other results, it was reasonable to assume that weakening of the Treg cell-mediated suppression would benefit both anti-cancer and neuroprotective immunity. It was recently shown that transforming growth factor (TGF)-β sustains the regulatory character of Treg (Fantini et al., 2004; Zheng et al., 2004) but no physiological compound has been identified that can weaken Treg. It is plausible that a physiological molecule capable of weakening the activity of Treg cells, at least in cases of CNS injury, must be a brain-derived compound whose half-life is short and whose concentration in the periphery is low.

Among the known neurotransmitters that participate in a stress response, are increased after injury, and are associated with tumors, dopamine seemed to us the most suitable candidate for interaction with Treg cells. Physiological dopamine is increased under stressful conditions (Saha et al., 2001; Vermetten and Bremner, 2002). Its peripheral concentration is low, it is highly unstable in the blood, and it has been shown to participate in neuroimmune dialog (Weihe et al., 1991; Robertson and Jain, 1995; Ilani et al., 2001; Levite et al., 2001; Lemmer et al., 2002). Almost all cells of the immune system, including T cells, bear dopamine receptors (Weihe et al., 1991). Interaction between neurotransmitters and immune cells (e.g. T cells) has been investigated in a whole population of T cells and in a subpopulation of CD4+ T cells, but no distinctions were made in those studies between regulatory and effector (autoimmune) T cells.

Studies of isolated populations of CD4 T cells, revealed that dopamine exerts a direct weakening effect on the suppressive ability of CD4+CD25+ (Treg) cells (Kipnis et al., 2004b). The receptors that mediate this effect belong to the type-1 family of dopamine receptors (D1R and D5R). These receptors are expressed only weakly, if at all, on effector T cells (Teff cells), but are strongly expressed on Treg cells, allowing preferential action of dopamine on Treg. Short-pulse application of dopamine to Treg significantly decreased the suppressive activity of Treg co-cultured with Teff. The inhibitory effect of dopamine on Treg suppressive activity was manifested in decreased production of interleukin (IL)-10 and TGF-β, which participate in cytokine-mediated suppression. Dopamine was also found to down-regulate CTLA-4, which is expressed constitutively on Treg and is responsible for cell-to-cell contact-mediated suppression. Other catecholamines, such as epinephrine, norepinephrine and serotonin, had no effect on Treg, although they affected Teff and their receptors were found to be expressed by Treg (Kipnis et al., 2004b).

The dopamine-induced weakening of Treg-cell suppressive activity might be considered a first signal in the cascade of events that activates the autoimmune T cells. With the suppression lifted, activation of Teff cells further requires presentation of their specific antigen and of a co-stimulatory molecule by APCs. The availability of antigen is apparently sufficient to maintain homeostasis in the normal healthy CNS, but after an acute injury or under chronic neurodegenerative conditions it appears that antigen availability on its own does not suffice. Thus, in the absence of a mechanism capable of weakening the suppressive effect of Treg cells, the evoked response might not be sufficient to cope with the demands generated by the injury (Schwartz and Kipnis, 2002).

In the case of malignancies, where autoimmunity is required to fight off the cancer, Treg cells interfere with the body’s natural propensity to activate a protective mechanism. Depletion of these cells, much as in the case of neurodegenerative conditions, increases the ability to reject tumors (Shimizu et al., 1999). In patients with certain malignancies, dopamine levels in the periphery are increased (Saha et al., 2001). It seems reasonable to suggest that this stress-related compound, possibly in combination with some other compound(s), might be the signal in cancer patients that weakens the Treg-mediated suppressive effect.

Dopamine or its specific D1 agonists have been shown to further block the Treg cell-mediated suppression of Teff cells (Kipnis et al., 2004b), a finding with intriguing implications for therapy. Injection of the D1 agonist SKF-38393, immediately after a traumatic injury, or induction of glutamate neurotoxicity in the mouse CNS, increases neuronal survival. SKF-38393 did not have any effect in mice devoid of T cells, indicating that its beneficial effect in the wild type was not exerted directly on the neurons, but rather via T cells. Since dopamine cannot cross the blood–brain barrier its effect in
wild type animals subjected to injury can be assumed to be peripheral. Treatment with dopamine and its relevant agonists might also be considered for cancer rejection. It was shown that regulatory T cells suppress a spontaneous T cell response to cancer and mice depleted of Treg efficiently reject transplanted tumors.

It should be kept in mind that T cells can do only what T cells can do regardless of their antigenic specificity. Production of cytokines and growth factors from T cells is a matter of their activation and taken within the context of the affected/injured tissue. Antigenic specificity of T cells could be viewed only as a means of homing T cells to a tissue/site in need. Once T cells reach the tissue in need and are reactivated, the antigenic specificity becomes irrelevant to the effector phase (Mizrahi et al., 2002). Therefore, in order to achieve any neuroprotective or other effect in the CNS, it would follow that T cells should be directed to CNS antigens. T cells do not have a way of directly communicating with neurons, as neurons do not express MHC class II molecules. Therefore, two routes of interactions are possible: either through cytokines produced by T cells following activation or through an indirect effect. The indirect effect could be mediated via microglia or astrocytes. Both of these cell types are able to express MHC molecules, whose expression is further increased following injury, thus enabling direct interaction of T cells with microglia (Moalem et al., 1999a). The role of microglia and astrocytes is far beyond what was originally thought. Glial cells do not only serve as glue for neuronal tissue but actively participate in excitation and neural firing and generally contribute to brain plasticity. Microglial cells, resident immune cells in the brain, are able to obtain various phenotypes, depending on activators and context of the tissue. Following injury and under chronic neurodegenerative conditions microglia might serve as a primary source for reactive oxygen species, glutamate, nitric oxide and other cytotoxic compounds (Rutkowski et al., 2000; Penkowa et al., 2003; Rostasy, 2005; Stadelmann et al., 2005). On the other hand, microglia, as well as astrocytes, have the potential to buffer glutamate and produce neuronal growth factors. Studies from our laboratory showed that T cells can affect the phenotype of microglia and switch it towards not only a less destructive but also towards a protective phenotype and even a phenotype that support cell renewal. Following activation of microglia with T cells, in-vitro, glutamate-buffering capacity of microglia is significantly increased, along with production of neuronal growth factors, e.g. BDNF, and there is a reduced expression of cytotoxic factors, e.g. TNF-alpha (Shaked et al., 2004; Butovsky et al., 2005a, b; Shaked et al., 2005; Butovsky et al., 2006).

Due to the high trafficking ability of T cells and their ability to penetrate tissues and “talk” with the local antigen-presenting cells, they can serve as mobile mini-factories with an ability to produce growth factors and cytokines upon need and thus maintain the homeostasis of the tissue. Under physiological conditions deviations are minimal and glial cells are able to maintain the homeostasis. Under pathological conditions, physiological activation of microglia cannot cope with the extreme deviation and thus T cells are required to facilitate glial activity to regain homeostasis. Therefore a dual effect of T cells in brain maintenance is achieved by prevention of a cytotoxic phenotype of glia and boosting of their protective phenotype (Schadlich et al., 1983; Schwartz, 2004; Schwartz and Kipnis, 2005b).

45.2.3. The Mechanism Underlying Protective Autoimmunity

There are many different subpopulations of CD4+ T cells, each responsible for a certain type of immune response. Th1 cells, for example, reinforce innate immunity and activate CD8+ T cells, whereas Th2 cells recruit and activate B cells. Autoimmune CD4+ T cells (Teff) locally boost and control resident microglia and infiltrating blood-borne monocytes, helping them to acquire the ability to fight off degenerative conditions requiring removal of dead cells and cell debris, as well as buffering of toxic compounds without producing inflammation-associated compounds such tumor-necrosis factor (TNF)-α, NO, or cyclooxygenase (COX)-2 (Levi et al., 1998; Basu et al., 2002; Janabi, 2002; Schwartz, 2003). Thus, according to recent results, the role of CD4+ T cells directed against self-antigens (helper T cells, Th) is to activate the innate response, enabling it to recognize that the threat to the tissue is not a harmful organism that it must kill, but as a toxic substance that it must neutralize or eliminate. In addition, the autoimmune T cells, upon encountering their specific antigens presented by antigen-presenting cells at the lesion site, can produce protective compounds such as growth factors and neurotrophins (Hammarberg et al., 2000; Moalem et al., 2000; Gielen et al., 2003). All of these tasks can be accomplished by a well-controlled response of helper T cells. These Th cells, in order to do their job, require local activation by their specific antigens residing in the site of stress. Thus, antigenic specificity apparently dictates the homing of T cells to the site where their local activation can occur. This is consistent with the findings that T cells having the same antigenic specificity are protective against different types of threatening stimuli occurring at the same site, or against different threatening stimuli at different sites occupied by the same immunodominant self-proteins. As a corollary, the same threatening stimulus, if manifested at different sites that do not share common dominant self-antigens, does not benefit from T cells directed against the same antigens.

Studies from several laboratories have shown that T cells patrol the healthy CNS, but do not accumulate there. The recent data suggest that, in the event of an acute injury or chronic neurodegenerative condition, T cells are recruited by and accumulate in the CNS (Hirschberg et al., 1998; Moalem et al., 1999a), where they might rescue neurons from degeneration if the damage caused by the toxic biochemical environment is not yet irreversible; moreover, the recruited T cells will prevent further deterioration. It is also possible that this autoimmune protective mechanism also operates when the
threat to the tissue is from microbial infiltration. In such a case the anti-self response might occur without the individual being cognizant of the response taking place, unless the harnessed autoimmunity gets out of control, in which case its effect is no longer beneficial but destructive, and might result in an autoimmune disease (Dal Canto et al., 2000; Miller et al., 2001). This might be the situation in individuals who are predisposed to autoimmune disease development (Kipnis et al., 2001). According to this theory the pathogenic self-proteins that have been implicated in autoimmune diseases are the very proteins against which a well-controlled T cell response is protective. This might help explain why autoimmune diseases are often attributed to viral infections in the brain. It might also explain the relatively low clinical prevalence of autoimmune diseases and their occurrence mainly in young adults rather than in the elderly population, whereas neurodegenerative diseases and cancer are common and significantly more prevalent in the elderly, in whom the immune system is deteriorating (Linton and Dorshkind, 2004).

45.2.4. The Missing Link—Adaptive Immunity Controls Microglia Phenotype Needed for Survival, Regrowth, and Renewal

It has long been believed that, irrespective of the type or context of an injurious stimulus, microglia show a stereotyped reaction in that they exhibit a predetermined program of executive functions. Experimental evidence supports the notion that some features of the microglial response might indeed originate in a core program of multi-purpose behavior. It should be noted, however, that most of what we know about the diverse activities of microglia emerges from in-vitro studies which cannot adequately reflect the complexity of microglial responses in vivo. Deprived of their physiological environment and triggered via a single receptor, the in-vitro response of isolated microglia is likely to be one-dimensional (Becher et al., 1996; Stalder et al., 1997; Lombardi et al., 1998; Smith et al., 1998; Kloss et al., 2001; Nakajima et al., 2001; He et al., 2002; Saura et al., 2003; Liuzzi et al., 2004; Shin et al., 2004; Vairano et al., 2004; Vegeto et al., 2004). As an example, when activated by bacterial components such as lipopolysaccharide, microglia acquire an inflammatory and cytotoxic phenotype (Lee et al., 1993; Merrill et al., 1993). This emergency scenario, in which the body’s fighting force is called upon to attack and kill bacteria, represents only one possible situation involving microglial activation. In contrast to the traditional notion of a stereotyped response, we favor the idea of diversity in microglial behavior. An acquired response that is defined and refined by an ensemble of incoming signals is not a new concept in cell biology. It does, however, represent a departure from the traditional view of microglia.

Danger signals can come from both foreign material (infectious agents) and endogenous sources (damaged cells or tissues, altered molecules, neurotransmitter imbalances). Endogenous toxicity (the ‘enemy within’ (Schwartz et al., 2003)) might result from membrane breakdown products, the extracellular presence of cytosolic compounds, abnormally processed or aggregated proteins (such as β-amyloid), or abnormal abundance of transmitters (such as glutamate). It appears that microglia fail to distinguish between external and self-derived enemies, and consequently their response to danger signals from endogenous agents resembles their response to invading microbes.

The microglial phenotype can be shaped by adaptive immunity (Butovsky et al., 2005b; Shaked et al., 2005). The chemotactic message of inducible microglial chemokines can be altered by a single T-cell cytokine: interferon (IFN)-γ, probably serving a feedback mechanism, alters the blend of chemokines, thereby shifting the preference for leukocyte subpopulations and conceivably affecting the composition of further infiltrates. Adding another piece to the puzzle, the nature and intensity of this response can be controlled by interleukin (IL)-4, a cytokine associated with Th2 cells (Butovsky et al., 2005b).

Thus, even standard responses triggered by established stimuli in simplified in-vitro settings show substantial variation when another factor is added. Microglia respond differently to the same stimulus if it co-exists with an additional stimulus, suggesting that they should be viewed as cells that acquire different phenotypes rather than behave stereotypically. Two stimuli can generate different effects, depending on the sequence of exposure: “priming” (preconditioning in which the first stimulus prepares the cell for an enhanced response to the second), negative priming (desensitization), or interference (where the second stimulus exercises a veto effect over an ongoing response to the prior stimulus). The two-signal interplay becomes even more complex when the time interval between the two exposures (manifested as ‘memory’) varies, as discussed below. This suggests that upon arrival of a modulator the executive functions of microglia can change not only in magnitude but also in quality. Variability of microglial activity is therefore not merely a reflection of stimulus strength or persistence, but is largely determined by the nature and context of the stimulus (Butovsky et al., 2005a, 2006).

In our view, microglia function as local sentinels. Under certain circumstances restricted activation of these stand-by immune cells might occur without being detected. If these sentinels fail to correctly read incoming stress signals, however, they will not develop the phenotype needed to fight off the threat, or alternatively, the cost of fighting off the threat is likely to outweigh the benefit (in terms of death of neighboring neurons). In addition, the outcome of a correct response to a particular signal could be detrimental if the signal itself is misleading. Self-compounds such as aggregated β-amyloid, for example, induce microglia to respond to them as if they were invading microorganisms to be killed. The phenotype utilized for that purpose is characterized by the production of cytotoxic molecules in quantities that the brain cannot tolerate. In addition, microglia that encounter, for example, aggregated β-amyloid fail to express class II major histocom-
patibility complex (MHC-II) molecules, and therefore lack the ability to interact locally with T cells (adaptive immunity) in the way needed for T cell-mediated expression of protective activity against local threats such as oxidative imbalance or cytotoxicity of neurotransmitters. Thus, paradoxically, under such conditions the microglia are precluded from participating in the adaptive immune responses needed to rescue the tissue from the toxicity that they themselves had helped to generate (Shaked et al., 2004, 2005).

Recent studies have shown that an inflammation-associated autotoxic phenotype not only causes neuronal loss but also interferes with neuronal survival, obstructs neurogenesis, and prevents regeneration (Butovsky et al., 2005a, b, 2006). In contrast, microglia that encounter adaptive immunity acquire a phenotype capable of presenting antigens and engaging in dialog with T cells. Such microglia, depending on the nature and amount of T cell-derived cytokines that they encounter, can become activated without producing the potentially cytotoxic cytokine tumor necrosis factor (TNF-α) or can even down-regulate its production. For example, operating via relatively small amounts of IFN-γ, the T-helper (Th)1 cells—classically viewed as pro-inflammatory—can activate microglia to buffer glutamate, a common player in neurodegenerative diseases (Shaked et al., 2005). Likewise, Th2 cells, commonly viewed as anti-inflammatory, by operating via IL-4 can activate microglia to produce insulin-like growth factor (IGF)-I (Butovsky et al., 2005b), known to be associated with cell renewal (Mattson et al., 2004; Varela-Nieto et al., 2004; Shetty et al., 2005; Sonntag et al., 2005; Butovsky et al., 2006). Microglia activated by IFN-γ or IL-4 therefore protect neurons and can support both neurogenesis and oligodendro-genesis (Butovsky et al., 2006).

We maintain that the protective versus destructive dichotomy of microglial effects does not necessarily reflect conflicting or contradictory activities. In normal healthy individuals, these cells stand ready and waiting to perform neural or immune tasks. However, because the CNS has a limited ability of the to tolerate any deviation from homeostasis, even defensive activity on the part of activated microglia can exacerbate a chaotic situation rather than resolve it.

Strategies for preventing overshooting of microglial reactions can be based on pharmacology, by employing selective suppression of undesirable activities while still permitting other executive functions to be performed. An alternative approach of recruiting T cells of a certain phenotype directed against weak agonists of self-antigens, might result in immunomodulation. Examples of such antigens are altered peptide ligands or the synthetic oligopeptide copolymer 1 (Cop-1) (Teitelbaum et al., 1997; Sela, 1999, 2000). By the use of such antigens for vaccination in a context of neurodegenerative conditions, it was possible—irrespective of the primary risk factor—to boost activity in a well-controlled way (Kipnis et al., 2000; Schori et al., 2001; Schwartz, 2001b; Schwartz and Kipnis, 2005a). Paralysis of microglia can be helpful within an experimental setting of a disease model. However, global depletion of microglia for extended periods might impair rather than preserve the structure and function of the CNS.

45.3. Development of Therapeutic Vaccinations

Any immune manipulation, which activates the immune system to induce a well-controlled increase in the likelihood that relevant T cells will home to a site of injury site, can be expected to be beneficial. Three major approaches could be considered: immunization with self-antigen agonists, induction of lymphopenia or functional inactivation of naturally occurring regulatory CD4+CD25+ T cells.

45.3.1. Immune-Based Vaccination for Neurodegenerative Diseases

45.3.1.1. Copolymer-1 (Glatiramer acetate, CA)

Recognizing that T cells are needed for assisting CNS in fighting off neurodegenerative conditions have prompted us to search for safe ways to do so without imposing the risk of developing autoimmune diseases. Data accumulated over the last decade has raised several options, including local transplantation of specially activated macrophages in cases of acute insult. In other situations of either chronic or acute neurodegeneration, the choice depends on the therapeutic window and the condition of the tissue (the critical issue being the bias of the microglia). In searching for active vaccination we considered using agonists of self-antigens. Such agonists can activate a response that weakly cross-reacts with the resident self-antigens. One such antigen is the copolymer glatiramer acetate, known as Cop-1, which is safely used daily for treating multiple sclerosis. Yet, for neuroprotection in cases of non-inflammatory neurodegenerative diseases the outcome was critically affected by the dosing, regimen and the choice of the carrier (adjuvant). In the case of animal model of ALS, the use of GA emulsified in complete Freunds adjuvant was beneficial, yet adjuvant-free GA was not found to be effective in any of the tested regimens (Haenggeli et al. 2007) and its daily administration was found to be destructive in female ALS mice (Bukshpan et al., unpublished observations). In a model of glaucoma, weekly or monthly injections of adjuvant-free GA were found to be beneficial, but not daily injections (Buklash et al. 2005). In an animal model of Alzheimers disease, a weekly injection was found to be beneficial (Butovsky et al. 2006b). Yet translating it into a human therapy requires careful determination of the regimen, which critically determines the T-cell phenotype (Th1, Th2, Treg); and thus, the clinical outcome.

45.3.1.2. Lymphopenia

Induction of lymphopenia significantly increases immunoreactivity towards cancer-specific proteins and efficiently sup-
presses cancer (Dummer et al., 2002). A sudden drop in the pool of peripheral T lymphocytes stimulates their homeostasis-driven proliferation in order to restore the pool. In response to the stimulus of lymphopenia, naive peripheral T cells proliferate and acquire a phenotype reminiscent of memory T cells (Ma et al., 2003). The induced proliferation predisposes the individual to development of an autoimmune response, since under lymphopenic conditions T cells can proliferate upon interaction with MHC-II molecules alone, with no need for a co-stimulatory signal (Sara et al., 1999; Gudmundsdottir and Turka, 2001; Elflein et al., 2003). If at the time of lymphopenia induction the body undergoes stress and consequently certain self-antigens are exposed (e.g., antigens related to tissue injury or cancer), an autoimmune response to these antigens will occur, resulting in a high overall incidence of the proliferation of the relevant T lymphocytes (Gelinas and Martinoli, 2002). In rodents suffering from acute or chronic neurodegenerative conditions, induction of lymphopenia significantly benefits post-injury neuronal survival (Kipnis et al., 2004b). Lymphopenia and the subsequent homeostatic proliferation can be induced in a number of ways, the most clinically relevant being low-dose irradiation of the lymphoid organs. As a result of the lymphopenia, T cells proliferate and become activated. They patrol the body, and their patrol route includes the CNS. On reaching the lesion site, and after being activated by the resident cells that present self-antigens in the MHC-II groove, these lymphopenia-derived T cells perform their effector functions, similarly to T cells obtained by immunization with self- or altered self-proteins (Kipnis et al., 2004a).

45.3.1.3. Attenuation of Regulatory T Cell Network

Since the aim is to achieve activation of T cells that cross-react with self-antigens at the site of injury, this can also be done by weakening the naturally occurring Treg cells. In an experimental context, nude mice (devoid of mature T cells) repopulated with a T cell population that did not contain the Treg-cell subpopulation (Kipnis et al., 2002) showed better recovery from a CNS insult than wild-type mice of the same strain. For clinical use, however, what is needed is a reagent that will weaken Treg cells. Dopamine, as mentioned earlier, was found to weaken both the activity and reduce the trafficking of Treg cells (Kipnis et al., 2004b). It is possible that dopamine represents a family of physiological compounds capable of controlling Treg-cell activity, therefore allowing speedy recruitment of the relevant autoimmune T cells. Development of synthetic compounds that can reproduce the dopamine effect is another apparently feasible approach in which a common immune-based therapy could be used to fight off neurodegenerative diseases, irrespective of etiology. Although such compounds might weaken Treg cells nonselectively (i.e., regardless of their antigenic specificity), the subsequently evoked autoimmunity will be restricted to CD4+ cells that encounter their relevant antigens, and will consequently be associated with the site under stress.

Summary

Harnessing the immune system in a well-controlled way might be the therapy of choice for neurodegenerative disorders. As long as the integrity of the immune system is maintained and neurotransmitter imbalance in the brain is within the remediable capacity of the immune system, homeostasis remains intact and the integrity of brain performance is preserved. According to this view, therefore, the widening age-related gap between deteriorating immunity and risk factors for diseases can be narrowed by appropriate activation of the immune system.

Review Questions/Problems

1. Absence of macrophages increases the rate of Wallerian degeneration
   YES/NO/UNRESOLVED
2. Lack of neuroprotection in thymectomized rats points to the role of naturally occurring regulatory CD4+CD25+ T cells in endogenous neuroprotection
   YES/NO/UNRESOLVED
3. Tova cells are not protective following CNS injury but will be protective following PNS injury
   YES/NO/UNRESOLVED
4. T cell specific to NoGo protein might induce neuroprotection in injured CNS
   YES/NO/UNRESOLVED
5. Absence of macrophages improves neuronal survival following PNS injury
   YES/NO/UNRESOLVED
6. Optic-nerve activated macrophages are better phagocytes than sciatic-nerve activated macrophages
   YES/NO/UNRESOLVED
7. Lymphatic drainage from the CNS is unique compared to lymphatic drainage from other tissues
   YES/NO/UNRESOLVED
8. GAP43 is a good marker for sprouting
   YES/NO/UNRESOLVED
9. Neuroprotection and regeneration are similar processes driven by different cell types
   YES/NO/UNRESOLVED
10. Perivascular macrophages are important “partners” in T cell-CNS interaction
    YES/NO/UNRESOLVED
11. Prompt autoimmune response to injury will induce neuroprotection only if this response is tightly controlled.

YES/NO/UNRESOLVED

12. Survival of neurons in the injured CNS is an outcome, at least in part, of protective immune response and a destructive local effect of physiological compounds that exceed their normal concentration.

YES/NO/UNRESOLVED

13. Only EAE-inducing TMBP cells confer neuroprotection following injury

YES/NO/UNRESOLVED

14. T cell specific to NoGo might induce neuroprotection in injured CNS

YES/NO/UNRESOLVED

15. Autoimmune T cell-based therapy can be easily combined with methylprednisolone

YES/NO/UNRESOLVED

16. Which one of the following statements is the most unlikely?
   a. Dopamine may affect CD4+ T cells
   b. Dopamine may affect Treg cells
   c. Treatment with Dopamine might exacerbate cancer
   d. Treatment with Dopamine might exacerbate autoimmune disease

17. Wild type Balb/c mice were injected with autologous carcinoma cells. One group was treated with Dopamine and the other with PBS. Mice are euthanized when cancer reaches 2 cm in size. The plot of survival of mice as a function of time is presented below. Which of the following statements is correct?

![Graph showing survival of mice as a function of time]

a. Group 1 was injected with PBS and group 2 with Dopamine
b. Group 1 was injected with Dopamine and group 2 with PBS
c. Additional information is required to address this question
d. None of the above

18. Multiple sclerosis patient was treated with a new cellular therapy – activated CD4+ T cells from this patient were isolated (based on the selection for CD25+), anergized in-vitro on immature dendritic cells and returned to the patient’s blood stream. This patient has also colon cancer. Which one of the following statements is the most likely?
   a. The treatment may exacerbate the ongoing MS disease
   b. The treatment may exacerbate the ongoing colon cancer
   c. The treatment will not affect any of the conditions
   d. The treatment will benefit both diseases

19. Neuroimmunology primarily deals with:
   a. Autoimmune inflammations in the brain
   b. Autoimmune inflammations in the injured brain
   c. Inflammatory pain
   d. The effect of mood on activation of T lymphocytes
   e. All of the above

20. Patient was treated with Copaxone for multiple sclerosis and has developed breast cancer. Doctors analyzed the blood samples and found an increase in regulatory T cell numbers based on CD25 and Foxp3 markers. The suggestion made by doctors was that Copaxone induces regulatory T cells and this may lead to cancer development. Which of the comments below is FALSE.
   a. Doctors might be right
   b. Doctors did not review the recent literature on Foxp3 in human Treg
   c. Only if these cells did not produce IL-2 they could be considered as Treg cells
   d. The suppressive activity of these cells in-vitro should be examined before addressing them a suppressor function
   e. All of the above

References


Hauben E, Butovsky O, Nevo U, Yoles E, Moalem G, Agranov, Mor F, Leibowitz-Amit R, Pevsner E, Akselrod S, Neeman M, Cohen IR, Schwartz M (2000b) Passive or active immunization with myelin basic protein promotes recovery from spinal cord con-


