Molecular Neurobiology

ISSN 0893–7648/06/34(3): 221–242/$30.00
ISSN (Online) 1559–1182

Molecular and Cellular Immune Mediators of Neuroprotection

Nicolas P. Turrin and Serge Rivest

Laboratory of Molecular Endocrinology, CHUL Research Center and Department of Anatomy and Physiology, Laval University, Québec, Canada

Abstract

Our view of the immune privileged status of the brain has dramatically changed during the past two decades. Even though systemic immune stimuli have the ability to activate different populations of neurons, cells of monocytic lineage also have access to the neuronal tissue and populate it as microglia. Although such a phenomenon is limited in intact brains, it is greatly increased during neurodegenerative processes associated with innate immunity and the release of pro-inflammatory molecules by either resident microglia or those derived from the bone marrow stem cells. The role of these events is currently a matter of great debate and controversy, especially as it relates to brain protection, repair, or further injury. In recent years, accumulating data have supported the notion that when immune molecules are timely released by microglia, they limit neuronal injury in the presence of pathogens and toxic agents, help clear debris from degenerated cells, and restore the cerebral environment for repair. It has been shown that alteration of the natural innate immune response by microglia has direct consequences in exacerbating the damages following acute injury to neurons. This article presents and discusses these data, supporting a powerful neuroprotective role for microglia and their innate immune reactions in response to pathogens and central nervous system insults.

Index Entries: Cytokines; inflammation; innate immunity; neurotrophins; neurodegeneration; demyelination; repair.

Introduction

The central nervous system (CNS) offers one of the greatest immune puzzles for the body; because of its indispensable functions, there is no margin for error in protecting it from pathogens, degenerative diseases, and trauma. Therefore, the immune system must act swiftly to eradicate any threats to the brain's function and integrity. However, because neurons cannot regenerate themselves and the brain's organization is complexly brittle, an immune

Received June 22, 2006; Accepted August 8, 2006.
*Author to whom correspondence and reprint requests should be addressed. E-mail: nicolas.turrin@crchul.ulaval.ca

response in its midst must remain as contained and inconspicuous as possible. In the case that the CNS is compromised and neuronal death is imminent, intrinsic mechanisms of repair and protection must be in place to minimize the damage and preserve neural tissue. This article addresses some of the recent findings that have elucidated immune-mediated mechanisms devised by the brain to protect itself from damage caused by various pathological and traumatic threats. The nature of the various causes of CNS damage are described as well as the different defense mechanisms available to the brain, including a description of the blood–brain barrier (BBB) and the innate and adaptive immune responses. Neuroprotection is then discussed, focusing on the endogenous cellular and soluble effectors involved in regulating neuroprotective processes in the CNS and various experimental models in which these events were discovered.

The Brain Under Attack

Dealers of Neuronal Death in the Brain

The fate of neurons and oligodendrocytes can be compromised by various insults and disease processes; some are acute, such as hypoxia, whereas others are more chronic, such as amyotrophic lateral sclerosis (ALS). An acute injury to the brain can set the stage for a graver neurological disorder, which could cause further damage to the CNS. For example, the neuronal death from a single febrile seizure episode could increase the preponderance for recurrent seizures and epilepsy. Furthermore, the damage caused by the insults can either be localized, as in the case of Parkinson's disease, where only the dopaminergic neurons of the substantia nigra are targeted. In other cases, the damaged areas can be less specific, such as in multiple sclerosis (MS), where demyelination appears somewhat arbitrarily in various areas of the CNS. Obviously, localized insults can be exacerbated and spread from beyond their initial point of damage. This phenomenon is exemplified by ischemia, where the primary infarct site is followed by a secondary area of neuronal death, known as the penumbra, which encompasses a far greater area of neurodegeneration than the original focal point *(1,2)*.

Some of the insults that target the CNS can directly kill neurons: neurons can be shredded by sharp objects or bone shards, perforated by bacterial toxins, deprived of energy stores, or overrun by viral particles. However, even if there are many exogenous insults that target neurons (pathogens, toxins, trauma, etc.), it is important to remember that most do not directly kill the cells of the CNS *per se*. Instead, they coax the neuron into initiating the intracellular chain of events that leads to death. This phenomenon occurs in cells that have been irreparably injured (e.g., a crushed or sectioned axon), deprived of energy or oxygen (as in the case of strokes), overstimulated (during excitotoxicity), or damaged by any other threat that terminally challenges the integrity of the neuron.

How Brain Cell Death Occurs

Irrespective to whether the cause of the neuronal death is known, the mechanisms by which neurons die have been well characterized. Factors that lead to the death of neurons include excess calcium influx, intracellular protein aggregates, oxidative stress, and the action of excitatory amino acid neurotransmitters, such as glutamate. Generally, neuronal death occurs with the cooperation of multiple factors—for example, excitotoxicity causes an overstimulation of the glutamate *N*-methyl-D-aspartate (NMDA) receptors, leading to the disruption of calcium homeostasis. In ALS, there is an agglomeration of certain glutamate transporters as well as deficits in glutathione peroxidase or superoxide dismutase action, both of which are anti-oxidant agents.

Depending on its intensity and chronicity, neuronal cell death is further categorized as either necrotic or apoptotic. Necrosis involves the rapid swelling and rupture of the neuronal

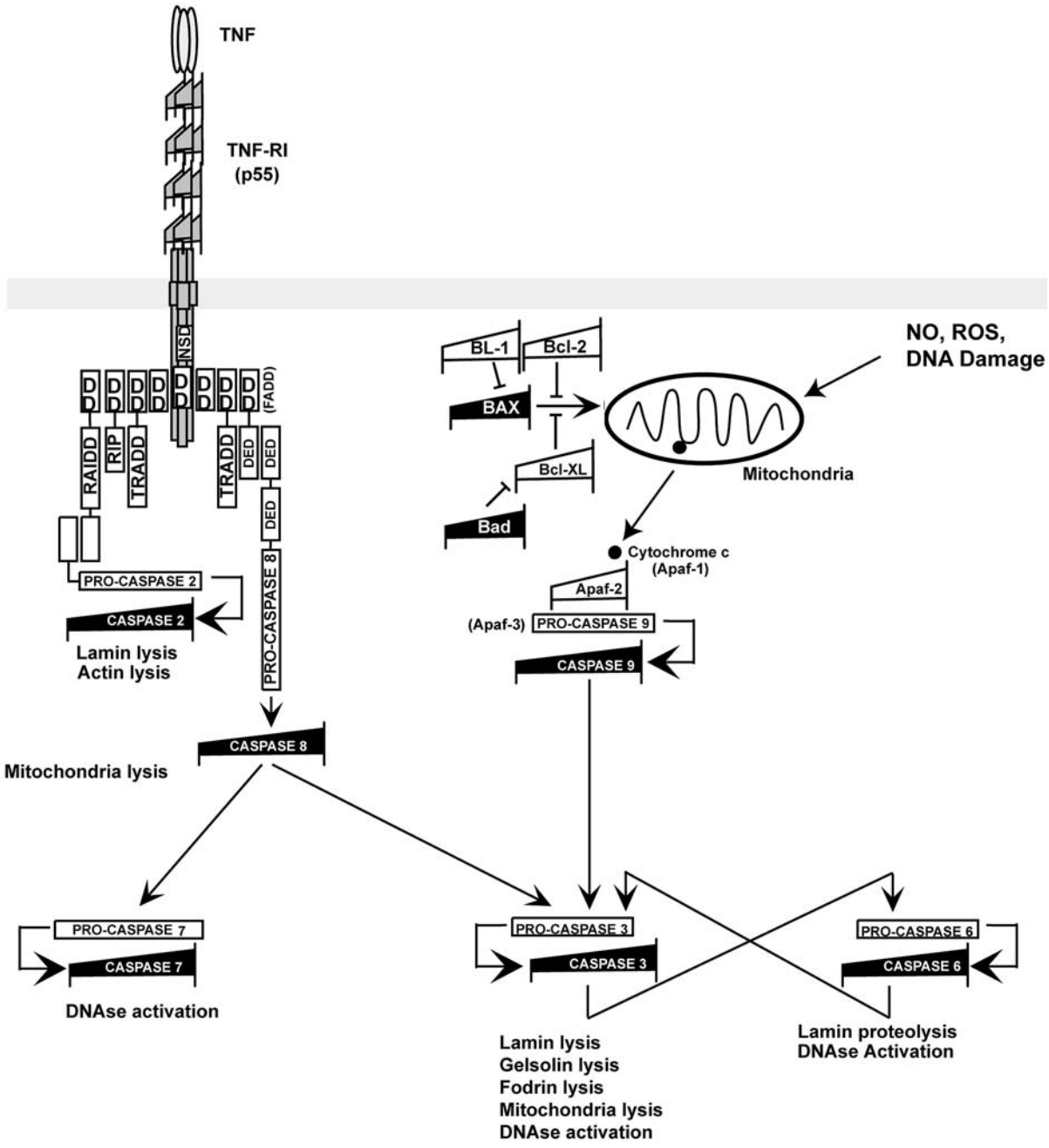


Fig. 1. Apoptosis signaling through death receptors and mitochondrial pathways. Tumor necrosis factor (TNF)-α binds to its type 1 receptor (TNF-R1), which is associated to a death domain comprised of TRADD), receptor interacting protein (RIP), RIP-associated ICH-1/CED-3 homologous protein with a death domain (RAIDD) and pro-caspases 2 and 8 to form a DISC), activating caspase 8. The initiator caspase 8 then activates the executor caspase 3. The mitochondrial pathway is initiated through oxidative stress, hydrogen reactive species and DNA damage and lead to the release of cytochrome *c*, which acts as the adaptor that couples to the apoptotic protease activating factor-1 (Apaf-1) and procaspase-9, forming the apoptosome complex during caspase-3 activation. Once activated, the executor caspase 3 precipitates the death of the cell through multiple mechanisms including DNA degradation and RNA splicing.

cell wall, whereas in apoptosis, the integrity of the cell is preserved until it is wholly removed by phagocytic cells (e.g., microglia) *(3)*. Furthermore, autophagy (the adaptive lysosomal process by which protein and organelles are turned over) can also cause neuronal death if it is defective or inefficient (*4*). During acute neurodegenerative processes, such as epileptic seizures and ischemia, the preliminary cell death is necrotic, because an excessively fast calcium influx into the cells causes them to lyse. However, in the secondary cell death regions of these conditions (such as the penumbra of an ischemic site) or during more progressive neurodegenerative processes, apoptosis prevails *(5)*.

The initiation of the apoptotic programmed cell death can be triggered through two pathways: through signaling of death receptors or through a mitochondria-mediated pathway. Both of these pathways ultimately lead to the activation of caspases (*see* Fig. 1). Death receptors belong to the tumor necrosis factor (TNF) gene superfamily, which includes the TNF receptor 1 (TNFR1) and CD95 (also known as Fas). Their signal transduction machinery induces the formation of the death-inducing signaling complex (DISC), formed by the association of the receptor, ligand (e.g., TNF-α and Fas ligand), and adaptor proteins (e.g., TNFR- or Fas-associated death domains [TRADD and FADD]). This is followed by the cleavage of caspase-8 and the activation of caspase-3, the final effector molecule that leads to the cell's demise.

On the other hand, oxidative stress, hydrogen reactive species, and DNA damage lead to mitochondrial apoptotic signaling, for which the main goal is the release of cytochrome *c*. Cytochrome *c* acts as the adaptor that couples to the apoptotic protease activating factor-1 (Apaf-1) and procaspase-9, forming the apoptosome complex during caspase-3 activation. The aforementioned caspases can be divided into two groups: initiator and executioner caspases. Caspase-8 and caspase-9 are initiator caspases that are activated through autocatalysis by interacting with adaptor proteins. Downstream caspases, such as caspase-3, are activated through their proteolytic cleavage by initiator caspases and are referred to as executioner caspases. Once activated, executioner caspases have several dozens of target substrates involved in DNA repair, messenger RNA (mRNA) splicing, and DNA replication (for a short review on apoptosis, *see* Ekshyyan and Aw *[6]*).

The Brain Fights Back

The BBB and Innate Immunity: The First Lines of Defense

The threats to the integrity of the CNS might appear numerous, but the brain is well-protected through physical as well as immunological means. First, the CNS is encased in a bony vault and the meninges (more specifically, in this case, the dura mater), protecting it from blunt trauma and invasive injuries. The brain is also bathing in cerebrospinal fluid (CSF), which dampens gravitational, rotational, and acceleratory forces. Additionally, the CNS is enriched in blood and macronutrients through an extensive vascular network.

The crosstalk between the brain and the blood is strictly regulated by the tight and adherent junctions that fasten the vascular endothelial cells of the brain microvasculature. The vessels are further covered by a basement membrane, and astrocytic end feet spread over the endothelia to act as ionic sinks and regulate the transport of proteins and macromolecules between the neurons and the blood. Finally, perivascular macrophages (or microglia) linger adjacent to the blood vessels to monitor the presence of pathogens or antigenic molecules. Together, all these cellular elements form what is referred to as the BBB *(7,8)* and provide a crucial line of defense between the brain and the rest of the body.

On the other hand, there are areas of the CNS that are deprived of a BBB—namely, the subfornical and subcommissural organs, organum vasculosum of the lamina terminalis, median eminence, neurohypophysis, pineal

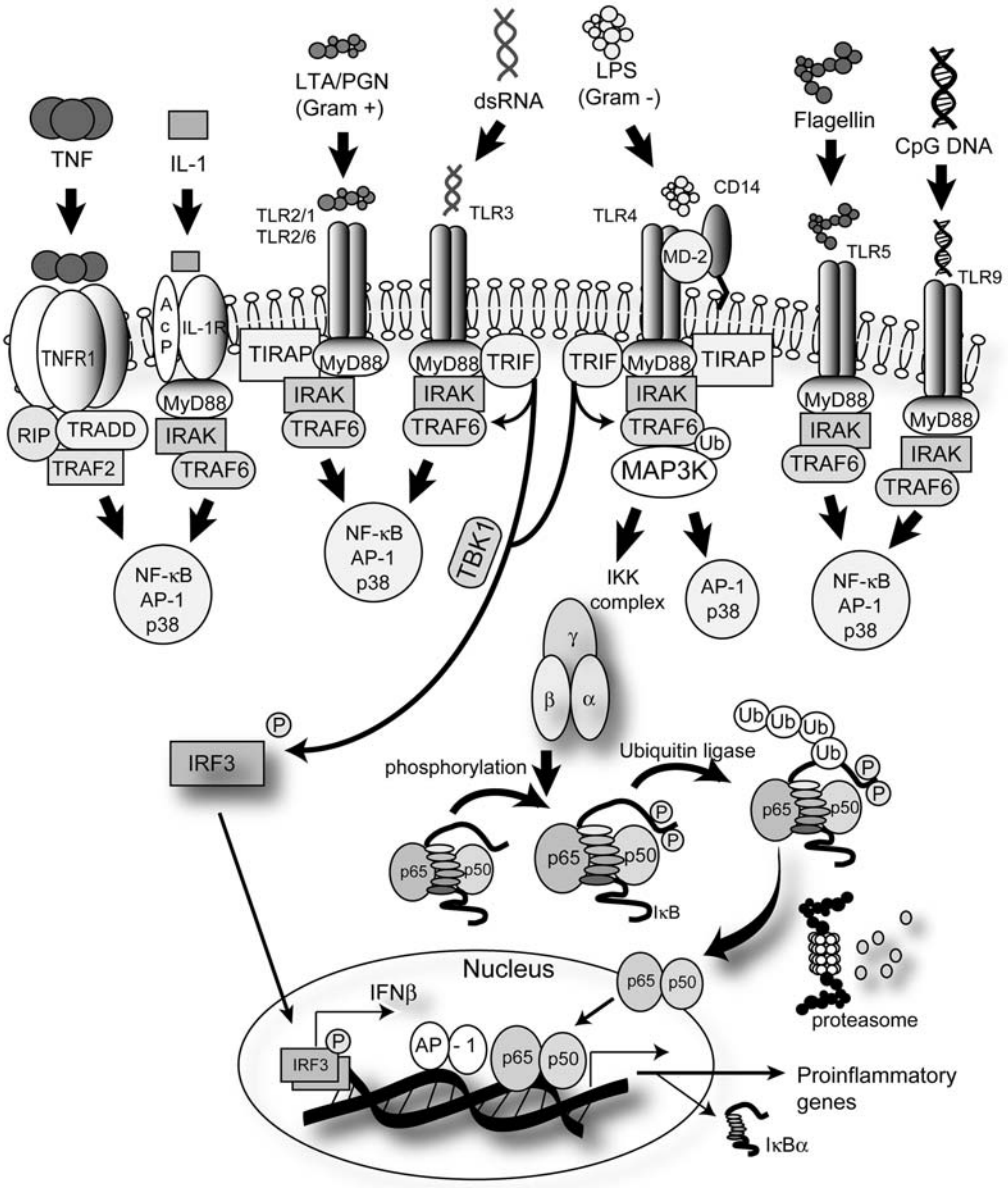


Fig. 2. Immune stimuli that trigger the synthesis of pro-inflammatory genes through NF-κB signaling. Endogenous molecules, such as the cytokines IL-1β and TNF-α, as well as the ligands for the various toll-like receptors (TLRs) trigger the separation of NF-κB from its inhibitory molecule (inhibitory κB; IκB) and its translocation to the nucleus of the cell. There, it binds to responsive elements in various pro-inflammatory genes and causes their transcriptional upregulation. NF-κB also promotes the transcription of its own inhibitor, IκB. Abbreviations: AcP, IL-1 receptor accessory protein; AP-1, activating protein-1; dsRNA, double-stranded RNA; IFN-β, interferon β; IKK, IκB kinase; IL-1R, IL-1 receptor; IRAK, IL-1R-associated kinase; IRF3, IFN regulatory factor; LPS, lipopolysaccharide; LTA, lipoteichoic acid; PGN, peptidoglycan; MAP3K, mitogen-activated protein kinase kinase kinase; RIP, receptor interacting protein; TBK-1, Traf family member-associated NF-κB activator-binding kinase 1; TIRAP, translation initiation region domain-containing adaptor protein; TNFR1, TNF-α receptor type 1; TRADD, TNFR1-associated death domain protein; TRAF, TNFR-associated factor; TRIF, TIR-domain-containing adaptor-inducing IFN-β; Ub, ubiquitin. (From ref. *70*. Copyright 2004 by Sage Publications. Reprinted by Permission of Sage Publications Inc.)

body, and area postrema, which are collectively referred to as the circumventricular organs (CVOs). The CVOs contain fenestrated capillaries and are mostly involved in the trafficking or monitoring of hormones and neuropeptides between the brain and the blood. The CVOs also act as major sites of immune mediation *(9,10)*.

Indeed, the CNS is able to mount an innate immune response during systemic infections, which originates from the CVOs and large blood vessels. To recognize the various threats to the brain, resident macrophages and microglia in the CVOs and perivascular monocytes express the innate immune Toll-like receptors (TLRs) *(11)*. This family of receptors recognizes pathogen-associated molecular patterns (PAMPs) without having primarily been exposed to them, thus initiating the first step of innate immunity, also referred to as inflammation. Indeed, the individual members of the TLR family (now numbering 13 in mice and 10 in humans) bind specific PAMPs either from bacteria (e.g., TLR2 from Gram-positive and TLR4 from Gram-negative bacteria), viruses (e.g., TLR3 responds to double-stranded RNA, whereas TLR9 recognizes the unmethylated CpG motif on DNA), or other pathogens (e.g., TLR6 dimerized with TLR2 can recognize mycoplasma). The cytoplasmic domains of TLRs share high homology with the interleukin-1 receptor (IL-1R) domain *(12)*. Therefore, it is not surprising that they associate with the adaptor protein MyD88 to activate the IL-1R-associated kinase (IRAK), ultimately leading to nuclear factor (NF)-κB signaling (*see* Fig. 2). In turn, NF-κB translocates to the nucleus of the target cell and triggers the expression of an array of pro-inflammatory genes, such as acute phase proteins, cytokines, chemokines, and the enzymes (e.g., cyclooxygenase-2 [COX-2], inducible nitric oxide synthase). Together, these molecules are involved in the initiation of the complex inflammatory response to immune threats.

Although the endothelia of the brain microvasculature are able to respond to circulating immune stimuli, the main cellular regulators of innate immunity in the brain are the microglia. These cells of myeloid lineage (which represent approx 5 to 10% of the adult brain cell population) are essentially the brain macrophages and the principal immune cells of the CNS. They can clear unwanted debris in the CNS by phagocytosis and produce many cytokines, making them part of the very elaborate innate immune system that serves to protect the nervous system from unwanted pathogens. It is believed that parenchymal microglia originate from pial macrophages and mesenchymal progenitors from the yolk sac, and they establish themselves in the brain during the embryonic stage *(13)*. However, recent data suggest the existence of two other subpopulations of microglial cells, each of which may have different origins. There is an ongoing debate regarding the maintenance of the microglial population in the adult CNS because more than 95% of all microglia are generated after birth and the formation of the BBB. One hypothesis states that adult microglia are maintained via self-replication or by the division of progenitor cells already present in the brain. Another hypothesis suggests that circulating monocytes are able to infiltrate the CNS and differentiate into microglial cells. Concrete evidence was recently obtained to demonstrate the capacity of bone marrow stem cells (BMSCs) to populate the CNS and differentiate into microglial cells. The principal method to study BMSC infiltration in the brain is to transplant bone marrow cells from a donor animal into an irradiated host animal. With the use of this model, many researchers have found donor-derived cells in the brain of host animals, and BMSCs do have the ability to populate the CNS and differentiate into functional parenchymal microglia and perivascular microglia *(14,15)*.

Although bone marrow-derived cells can enter the brain parenchyma throughout the CNS of normal mice, it appears that they are preferentially attracted to regions afflicted by neurodegeneration or neurological insults. In the case of cerebral ischemia, round donor-derived cells (most likely blood monocytes)

enter the brain at the site of injury. They then migrate from the infiltration site and become ramified microglial cells. This is also true in models where the BBB is not compromised, such as in the case of facial nerve axotomy *(16)*. In different models of brain injury, such recruitment is preceded by a rapid proliferation of resident microglia. These findings suggest the existence of various types of microglia in the injured CNS: (a) activated resident microglia; (b) newly differentiated microglia derived from resident progenitors; and (c) bone marrow-derived microglia that are able to enter the CNS even if the BBB is intact. The exact role of each subpopulation of microglia in the injured CNS remains unknown.

Interestingly, tissue macrophages express high levels of major histocompatibility complex (MHC) class II and are thus good antigen-presenting cells (APCs), whereas parenchymal microglia express low levels of this protein, indicating that they are poor APCs. However, it was recently shown that microglia originating from BMSCs expressed higher levels of MHC class II than their residential counterparts. This suggests that the infiltrated microglia are good APCs and better phagocytes. In mouse models of Alzheimer's disease, blood-derived microglial cells were found to be associated with amyloid plaques. These microglia are able to prevent the formation or eliminate the presence of amyloid deposits *(17)*. The newly recruited microglia are specifically attracted to the β-amyloid 40/42 isoforms in vivo and they participate in the elimination of these proteins by phagocytosis. Consequently, they may play a critical role in removing toxic elements from the CNS environment and restoring the milieu for recovery and repair.

The infiltrating phagocytes may actually enter damaged regions of the brain to clear the cellular debris left by dying cells and help re-establish a functional neuronal environment. To this end, inflammation may also contribute to restore the myelin sheath. There is a temporal expression of neurotrophic factors that follows the endogenous production of pro-inflammatory cytokines after cerebral injury, suggesting a role for the inflammatory response in mediating neurotrophism. Consequently, inhibiting specific inflammatory pathways may be suitable during demyelinating episodes but not during remyelination and repair. Therefore, it is crucial to determine the role of each type of microglia and define how these cells can be guided to decrease damages and improve recovery and repair. It is also important to mention that microglia are not the only glial cells involved in protecting the brain. Astrocytes and oligodendrocytes are also involved in crucial processes that support the role of microglia during inflammation and maintain the integrity of the CNS during immune and neuroprotective events. However, in depth discussion of the role of these glial cells is beyond the scope this review (*see* reviews by Dong and Benveniste *[18]* and Lubetzki et al. *[19]*).

Adaptive Immunity in the Central Nervous System

Microglial cells and infiltrating macrophages are able to process and present antigen. Logically, it follows that there is the possibility of a central adaptive immune response. Furthermore, microglia are able to produce IL-12, which is paramount in the transfer to adaptive immunity. However, if specific immunity is to occur, $CD4^+$ T-lymphocytes need to be present to recognize antigen and perpetuate this response. The presence of $CD4^+$ T-cells within the CSF has been documented, and the migration of T-cells from the blood to the CSF or parenchyma is possible into the normal brain. This phenomenon is greatly enhanced in the inflamed brain through a more permissive BBB and at privileged sites, such as the choroids plexus, CVOs, and subarachnoid space. This is possible through the interaction of cell-surface proteins such as intercellular and vascular cell adhesion molecules, as well as through the action of cytokines (especially chemokines) and their receptors (for a review, *see* Engelhardt and Ransohoff *[20]*). Therefore, the brain is able to mount an effective adaptive immune response but not without its drawbacks: the

switch to adaptive immunity could be at the heart of auto-immune central disorders, such as MS. In such a case, an immune response is mounted against self-antigens present in oligodendrocytes, thereby demyelinating neurons of the CNS *(21)*.

Immunity in the Brain: Too Much of a Good Thing?

In the past decade, increasing evidence has confirmed that the brain and the immune system are on much better terms that previously believed. The brain contains its own immune cells (the perivascular macrophages and the resident microglia) that are able to respond to damages and pathogens and initiate the proper innate and adaptive immune responses. This puts the CNS in an apparently more advantageous position to fight off injury and infection. However, it also makes it vulnerable to the collateral damage associated to a central immune response. For example, to eliminate pathogens or damaged cells, the innate immune process involves the release of reactive oxygen species and nitric oxide (NO). These might collaborate as noxious stimuli to healthy bystander neurons and glial cells if their delivery is not properly regulated *(22)*. Furthermore, if inflammation persists (e.g., through lack of a proper negative feedback by glucocorticoids) and the brain is overexposed to cytokines such as TNF-α, then devastating damage can occur *(23)*. The initiation of innate immunity in the brain also has a significant effect in the brain's vasculature: inflammation dilates the brain's vessels and causes hypotension; therefore, it can hinder the transport of nutrients and oxygen to the injured site. Furthermore, because it renders the BBB more malleable to permit the passage of immune cells into the CNS, there is also the possibility that harmful blood proteins (e.g., antibodies to self-antigens), or opportunistic pathogens may invade the brain to exacerbate the current condition *(24)*. Some of the same considerations must be given for adaptive immunity: humoral immunity in the brain involves the recruitment of lymphocytes that may aggressively kill pathogens and infected neurons and could cause collateral damage to the CNS. However, recent experiments have also postulated that antibodies have the ability to promote myelin repair in mouse models of MS *(25)*.

Neuroprotective Mechanisms in the Brain

Apparently, the immune system and CNS have developed a wide array of strategies to fight against pathogens and respond to trauma. This occurs because of the vast number of endogenous failsafe mechanisms integrated into the CNS immune response that promote a well-regulated response to injury and the initiation of recovery and repair. This section highlights the importance of letting most well-regulated immune responses in the brain run their course. It also describes how immune responses in the CNS promote not only the elimination of cerebral insults but the prevention of further damage and the recovery of the nervous tissue.

Neuroprotection and Innate Immunity

Because it represents the first response against most pathogens and injury to the CNS, inflammation often sets the pace for the immune events that follow it and determines the fate of the nervous tissue under attack. Consequently, innate immunity must be explosive; this is achieved by having the microglia recognize the injury or pathogen and quickly initiate production of pro-inflammatory molecules. These pro-inflammatory effectors mobilize the immune system, help recruit the appropriate cells to clear debris, and minimize primary neuronal damage and the secondary wave of neuronal death. If the innate response is hindered, then the initial damage done by a CNS threat could become overwhelming or pathogen load could reach levels that cannot be managed after. A recent experiment showed the importance of the acute innate response in

encouraging the recruitment of oligodendrocyte progenitor cells (OPCs) and, therefore, the remyelination process *(26)*. The enhancement of innate immunity by infusing lipopolysaccharide (LPS) endotoxin following a chemical lesion caused by ethidium bromide encouraged remyelination at the site of the lesion in the mouse brain. The study also showed that anti-inflammatory treatment using dexamethasone enlarged the size of the lesion caused by the surfactant Tween.

Another experiment demonstrated that alteration of the microglia response to an acute neuronal insult greatly exacerbated the damage in the CNS *(27)*. Indeed, mice lacking the proinflammatory cytokine TNF-α had a dampened initial activation of the microglia at the site of injury 6 h following an intrastriatal sodium nitroprusside (SNP) infusion, which was followed by profound neurodegeneration: the volume of neuronal death and demyelination caused by the NO donor SNP increased fivefold at day 4 postinjection compared to wild-type controls (ref. *27*; *see* Figs. 3 and 4).

The critical role of inflammation—especially for TNF-α—has been supported for both neuroprotective *(28)* and remyelination *(29)* processes. Notably, the kinetics of the TNF-α response are key to its neuroprotective role: In the model of SNP-induced excitotoxicity, the additional administration of TNF-α concomitantly with SNP exacerbated the damage and inflammatory response caused by the NO donor *(30)*. Furthermore, the chronic infusion of TNF-α into the brain by itself is able to cause neurodegeneration *(23)*. The dual role of this cytokine may be explained through activation of its different receptor subtypes. Dual and opposite roles have already been suggested for the TNFR1 and TNFR2 in mediating ischemic neurodegeneration *(31)*. TNFR1 is believed to be associated with the apoptotic effects of TNF-α, which appear to be dominant in models where TNF-α production is overstimulated (chronic TNF-α brain infusions, auto-immune diseases, etc.). On the other hand, TNFR2 is associated with neuroprotective effects through a Akt-1/PKB-dependent pathway *(31)* and appears to promote neurotrophic and remyelination mechanisms. Akt-1 has neuroprotective properties by stabilizing the mitochondrial membrane potential and preventing the release of cytochrome c, thus inhibiting apoptosis *(32)*. Guo and colleagues *(33)* emphasized the importance of TNF signaling in the brain during excitotoxicity using chimeric mice in which the bone marrow-derived cells (which included infiltrating macrophages and microglia) were replaced with cells lacking both TNF-α receptors. These mice were found to be more vulnerable to excitotoxic damage than their nonchimeric counterparts. Furthermore, Arnett and colleagues *(29)* clearly showed a role for TNF-α and its type 2 receptor in the proliferation of oligodendrocytes and remyelination in an acute model of demyelination. Others have suggested that TNF-α-NMDA-dependent NF-κB activation through TNFR2 is essential to protect against glutamate-induced neurotoxicity *(28)*.

The neuroprotective role played by NF-κB and Akt-1 is further emphasized by other cytokines, such as stem cell factor (SCF), another cytokine that promotes neuroprotection through Akt, as well as the mitogen-activated protein kinase (MAPK) ERK *(34)*. Erythropoietin (Epo), which has only recently been characterized as a cytokine *(35)*, was first shown to have neuroprotective roles when it was supplemented following ischemic episodes *(36)*. It was later found to protect the brain and spinal cord during traumatic injuries *(37,38)* and in various other neuropathies (for a review, *see* Bartesaghi et al. *[35]*). Indeed, Epo was effective in treating patients suffering from stroke, decreasing infarct size and improving outcome *(39)*.

Similarly to TNF-α and SCF, some of the protective actions of Epo occur through its signaling via NF-κB- *(40)* and Akt *(41)*-dependent pathways. Epo can also signal through transcription factor signal transducers and activators of transcription (STAT)5, which mediates the induction of Bcl2 and Bcl-xL, both of which are inhibitors of apoptosis *(42)*. Epo also causes an intracellular calcium increase that is believed to be related to an increase in brain-derived

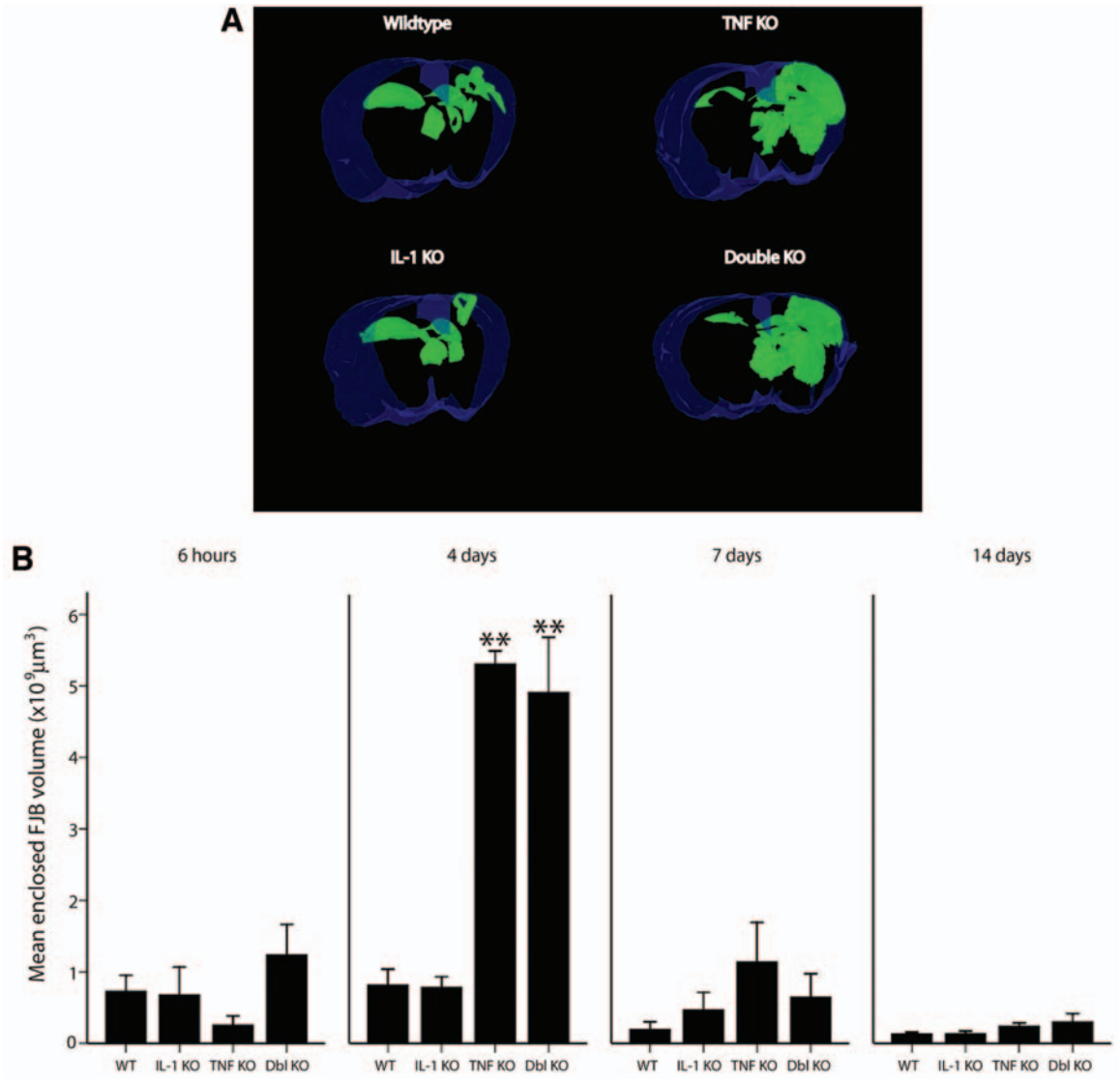
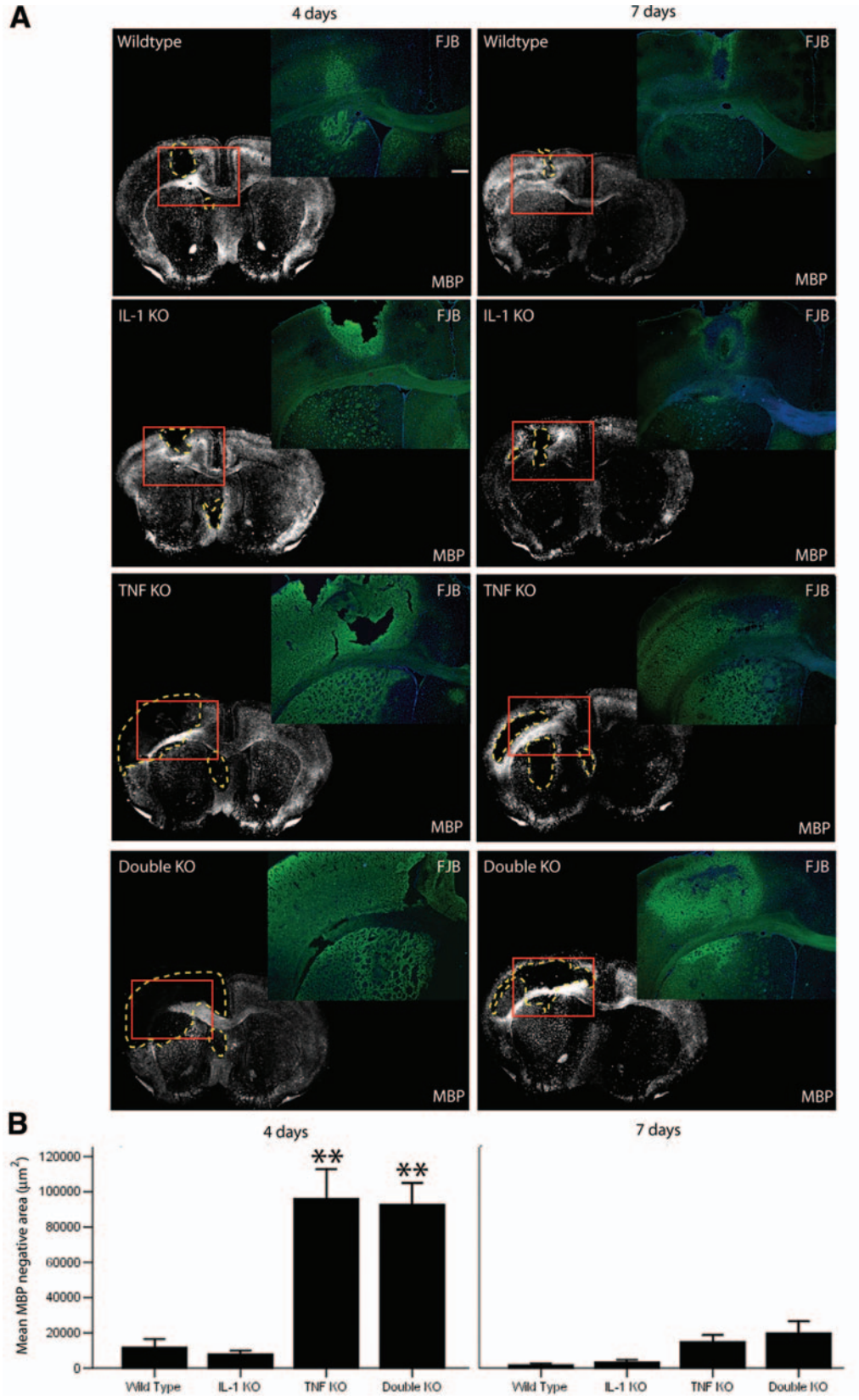


Fig. 3. Three-dimensional representation of the volume of neuronal cell death, as measured by Fluoro-Jade B (FJB)-positive staining in wild-type, IL-1β, TNF-α, and IL-1β/TNF-α double (Dbl) knockout (KO) mice killed 4 d after an intraparenchymal injection of the excitotoxin sodium nitroprusside. **(A)** These 3D reconstructions of FJB-positive regions were performed using a C-80 Nikon microscope and super-high pressure mercury lamp (Nikon, Montréal, QC) fitted with a Retiga EXi Fast digital camera (QImaging, Burnaby, BC) feeding to a Precision 660 workstation (Dell Computers, North York, ON). The FJB-positive regions were traced on a Wacom pen tablet (Wacom, Vancouver, WA) using Neurolucida stereological software package (v. 6. 02. 1, Microbrightfield, Williston, VT), and 3D reconstruction and volume determination were compiled using the Neuroexplorer software (v. 4. 01. 1, Microbrightfield, Williston, VT). Please note the larger volumes of FJB in the TNF and Dbl KO animals compared to that of wild-type and IL-1 KO mice. **(B)** Quantitative analysis of the FJB volume in the brain of wild-type, IL-1, TNF, and Dbl KO mice over time. Please note the rapid appearance of neuronal cell death 6 h following sodium nitroprusside injection and the marked spread of FJB staining (**$p < 0.01$ compared to wild-type and IL-1 KO, data presented as mean ± SEM) in the CNS of TNF and Dbl KO 4 d following the single sodium nitroprusside infusion. (Reproduced with permission of the Society for Neuroscience, copyright 2006.)

Fig. 4. Sodium nitroprusside is highly toxic for both neurons and oligodendrocytes in the brain of TNF-α-deficient mice. Expression of myelin basic protein (MBP) mRNA, and FJB staining in the brain of wild-type, IL-1β, TNF-α, and IL-1/TNF Dbl KO mice 4 and 7 d after an intraparenchymal sodium nitroprusside injection. Darkfield photographs of *in situ* hybridization of MBP transcript were taken from brain slices dipped into NTB emulsion milk (Kodak) exposed for 10 d. Insets show fluorescent photomicrographs from representative examples of FJB staining, used here as an index of neuronal death (green neurons). Both sections in each panel (MBP mRNA and FJB staining) came from the same mouse. Please note that although FJB signals increased across the hemispheres of TNF-α and Dbl KO mice, MBP hybridization signal essentially vanished in the same area. A decrease in MBP gene expression levels is indicative of demyelination levels (depicted by the dashed yellow areas), which is significantly higher in TNF and Dbl KO mice compared to the other groups of animals at time 4 d (**$p <$ 0.01, data presented as mean ± SEM). White scale bar = 250 µm. (Reproduced with permission of the Society for Neuroscience, copyright 2006.)


A
4 days
7 days
Wildtype
FJB
MBP
IL-1 KO
TNF KO
Double KO
B
4 days
7 days
Mean MBP negative area (µm²)
120000
100000
80000
60000
40000
20000
0
**
**
Wild Type
IL-1 KO
TNF KO
Double KO

neurotrophic factor (BDNF), which is also stimulated by Epo through Ca^{2+}/cyclic adenosine monophosphate (cAMP)-response element-binding protein (CREB) cellular signaling *(43)*. BDNF is part of the larger family of neurotrophic factors and is further discussed in the next section. Similarly, overexpression of macrophage colony-stimulating factor (M-CSF) in microglia enhances BDNF production and restricts the damage of excitoxicity *(44)*. M-CSF acts on microglia through its receptor (M-CSFR) and promotes the production of pro-inflammatory cytokines and increases their phagocytic activity, thereby promoting neural survival *(45,46)*.

Through its actions on its various receptors (namely, EP_1 through EP_4), PGE_2 is also known to act on the brain and influence the fate of neurons during various insults. An agonist of the EP_4 receptor was found to protect against NMDA excitotoxicity in mice *(47)*, and mice lacking the EP_2 receptor showed greater damage following NMDA injections *(48)* and ischemia *(49)*. Furthermore, PGE_2 actions through EP_2 and EP_3 have been found to protect motor neurons in a culture model of ALS through their respective G protein-dependent pathways *(50)*. Conversely, EP_1 signaling contributes to excitotoxicity, because EP_1-deficient mice exhibit smaller infarct from NMDA *(51)*, and EP_2-deficient mice have a decreased β-amyloid burden in mouse models of AD *(52)*.

The discrepancies between the actions of the different EP receptors on neuroprotection can largely result from their different location and role in the CNS *(9)*. Generally, the neuroprotective actions of PGs can be attributed to their strong induction of neurotrophins *(53)*. Lipid messengers other than PGs, such as docosahexaenoic acid, are also implicated in neuroprotection and employ similar anti-apoptotic pathways, as described earlier (for a review, *see* Bazan and colleagues *[54]*). Similarly to most of the pro-inflammatory molecules discussed in this section, PGE_2 appears to be neuroprotective in acute models of neurodegeneration, whereas the prolonged expression of the PG promotes chronic inflammation and the neuronal death associated with it. Therefore, as much as it is important to let the CNS initiate an innate immune response, it is essential that this response be well-modulated and shut down as quickly as possible.

Innate immunity is not only implicated in neuroprotection but also promotes regeneration of some specific axons, such as those of the optic nerve after it has been crushed *(55)*. In this case, macrophages neighboring the crushed axons promote their regeneration by synthesizing oncomodulin (OM), a calcium binding protein that promotes the regeneration of the mature rat optic nerve in presence of cAMP and mannose *(56)*. These data warrant consideration of OM as a legitimate and specific growth factor for the mature optic nerve, because other growth factors were not found promote regeneration as much as OM.

Neurotrophins: Promoters of Neuroprotection and Repair

As mentioned earlier, BDNF is part of a larger family of neurotrophins characterized by their tropomyosin-related tyrosine kinase (Trk) receptors, as well as activation of the p75 neurotrophin receptor ($p75^{NTR}$). There have been several in vivo studies confirming the essential function of Trk receptors (which act through Akt-1, MAPK-dependent pathways) and $p75^{NTR}$ (which acts mostly through NF-κB) in promoting neuronal survival as well as the importance of their spatiotemporal regulation for their protective role to occur (for a review, *see* Kalb *[57]*).

Neurotrophin-4/5 (NT-4/5) was shown to protect pyramidal cells in a model of traumatic brain injury *(58)*. Additionally, NT-4 (as well as BDNF) has anti-apoptotic effects on cerebellar neurons in response to the neurotoxin cytosine arabinoside *(59)*. Another neurotrophin, nerve growth factor, protects neurons against ischemic death and UV neurotoxicity through a $p75^{NTR}$-dependent mechanism *(60)*. Furthermore, some neurotrophins (namely, BDNF and insulin growth factor [IGF]-1) are able to attenuate blood–spinal cord barrier disruptions following spinal cord injuries *(61)*. However, similarly to

TNF-α and other pro-inflammatory cytokines, it is important to remember that these neutrophins can have dual effects on the brain, both neuroprotective and neurodegenerative.

It was once believed that all the death-promoting actions occurred through the $p75^{NTR}$ receptor, whereas the Trk receptors mediated the neuroprotective effects *(62)*. As demonstrated earlier, recent experiments have shown that this distinction is not as clear as was first theorized *(60)*, because beneficial effects of $p75^{NTR}$ activation and some detrimental effects for Trk receptors have been discovered *(57,63)*.

Another family of neurotrophic factors includes the members of the IL-6 superfamily, which are characterized by the presence of gp130 as part of their receptor complex. This family includes IL-6, ciliary neurotrophic factor (CNTF), and leukemia inhibitory factor (LIF). These cytokines signal via the STAT3 pathway, activate Akt-1 and Bcl-xL *(64)*, and have similar anti-apoptotic and neuroprotective activities as ligands for the Trk receptors. For example, CNTF-deficient mice show greater loss of GABAergic neurons following a septohippocampal axonal lesion *(65)*. Furthermore, T-cell-produced LIF could have a neuroprotective role in patients with MS by counteracting the aberrant TNF-α apoptotic signal noted in this disorder *(66)* and may help recovery in a mouse model of spinal cord injury *(67)*.

Finally, IL-6 interventions protected neurons and myelin from NMDA-induced excitotoxicity in hippocampal slices *(68)*. Together, the IL-6 superfamily is yet another strategy by which the distressed CNS can protect itself from apoptotic processes and initiate repair mechanisms.

Help From the Periphery: Steroid Hormones and Neuroprotection

The BBB enables the brain to have tight control over the trafficking of peripheral proteins and macromolecules into the CNS. However, there are essential hormones that still gain access to the CNS and provide invaluable feedback and regulation for the central immune response. First, the glucocorticoids (GCs) offer the greatest endogenous negative feedback on the immune response by signaling through their intracellular glucocorticoid and mineralocorticoid receptors. These nuclear receptors act directly on the promoter regions of immune genes to repress their transcription and the innate immune reaction *(69)*. The HPA axis response that culminates with these GC actions is crucial in modulating the length and intensity of the response by the CNS to inflammation (ref. *70*; *see* Fig. 5). As shown by our group, a well-regulated inflammatory response from the intraparenchymal LPS injection did not cause any cellular damage to the nervous tissue *(23)*. However, if GC feedback is inhibited by Mifepristone (RU486), the inflammatory response does not shut down and massive neuronal death and demyelination occurs as a result of the exaggerated TNF-α response *(23)*. Because of the ease by which steroids cross the BBB, the neuroprotective effects of GCs and their synthetic equivalents have been exploited therapeutically for MS *(71)* and postinfectious encephalomyelitis *(72)*, and they have shown promise in a mouse model of Parkinson's disease *(73)*. However, treatment of some other neurological damages with GCs or their synthetic equivalents has met with mixed results and concern. The efficacy of GCs or GC-like compounds for treating spinal cord injuries has often been overshadowed by major side effects *(74,75)*, and a meta-analysis of their use for treating acute traumatic brain injuries has shown greater mortality *(76)*. Together, this would suggest that interventions using exogenous GCs are more efficacious in protecting the brain against chronic inflammatory disorders. However, acute nervous injuries benefit from a regulated inflammatory response and should not be interrupted by exogenous GC treatments.

Estrogen has also emerged as playing neuroprotective roles in the brain by having antioxidant, anti-inflammatory, anti-apoptotic, and neurotrophic effects during various neurodegenerative conditions (for an extensive review,

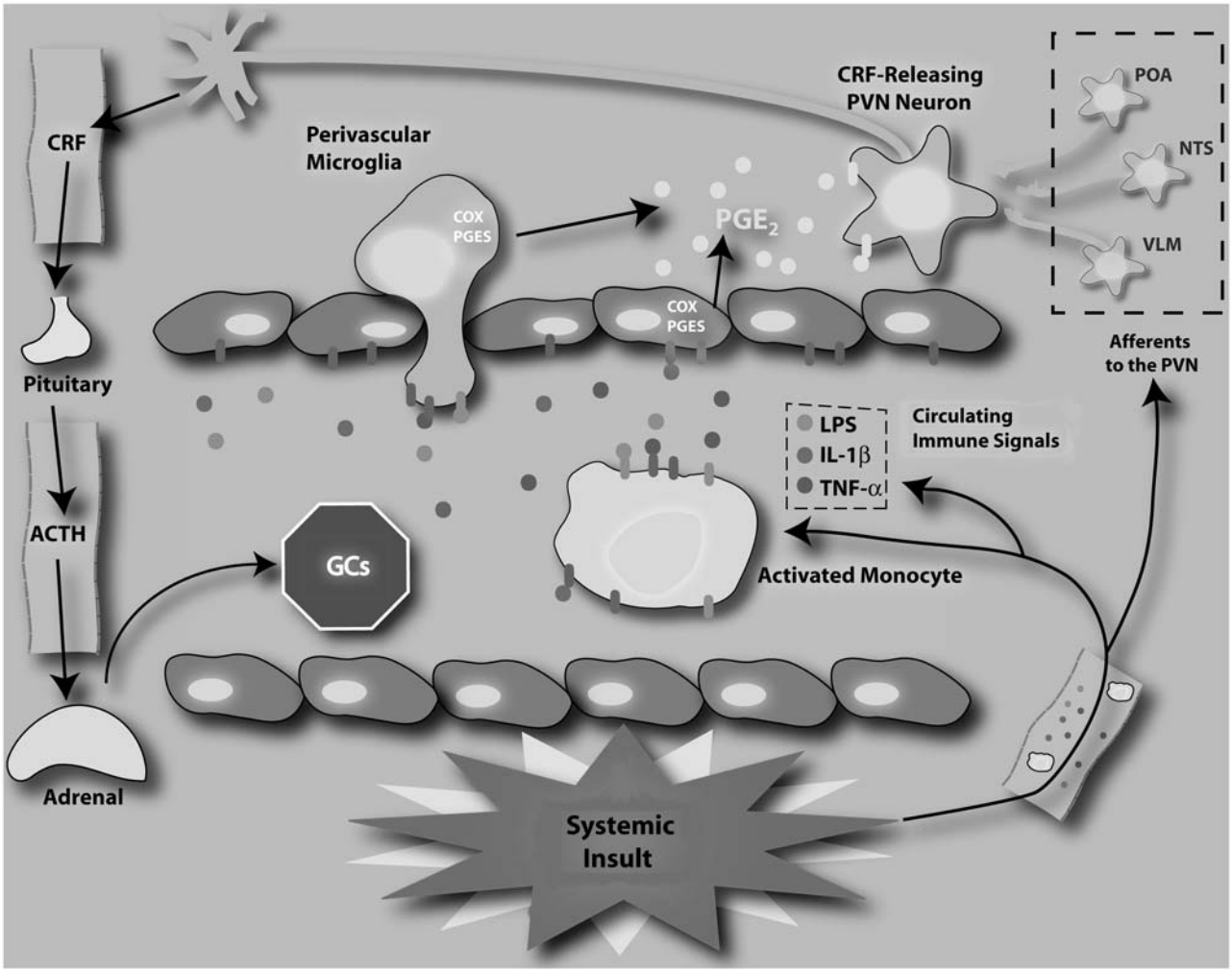


Fig. 5. Systemic immune reaction and the hypothalamic–pituitary–adrenal (HPA) axis. When an immune threat (in this case, lipopolysaccharide [LPS]) is present in the body, activated monocytes, and the pro-inflammatory cytokines (e.g., IL-1β, TNF-α), travel through the blood, along with LPS, and interact with the blood–brain barrier. The endothelial cells and the associated perivascular microglia synthesize prostaglandin E_2 (PGE_2) using cyclo-oxygeneases (COX) and prostaglandin E synthases (PGES). The PGE_2 is released in the parenchyma and acts on the corticotropin-releasing factor (CRF)-secreting neurons of the paraventricular nucleus of the hypothalamus (PVN). The CRF enters the portal circulation and reaches the adenohypophysis part of the pituitary gland and causes the release of adrenocorticotropic hormone (ACTH) into the systemic circulation. ACTH then reaches the adrenal gland and triggers the synthesis and release of glucocorticoids (GCs), which may act as negative feedback on the inflammatory response. A similar response occurs at distal sites that project to the PVN (pre-optic area), nucleus of the solitary tract, ventrolateral nucleus of the medulla. Activation of these circuits results in an integrated response to immune stimuli, which are directly under the control of the final product of this neuroendocrine axis, GCs. (Used with permission of the Society for Experimental Biology and Medicine, copyright 2004.)

see Amantea et al. *[77]*). In ischemia, not only does estrogen exert these effects, but it also preserves the microvascular blood flow at the site of the ischemic injury, preventing hypoxia *(78)*. The anti-apoptotic action of estrogens is believed to occur through prevention of a decrease of Bcl-2 at the site of injury *(79)*, and these effects occur mostly through the estrogen receptor-α (ER-α). This receptor can activate most of the anti-apoptotic pathways mentioned in earlier sections (e.g., Akt, CREB, NF-κB, ERK) *(80)*. Activation of the ER-α has also been effective in preserving dopaminergic functions in the rat nigrostriatal system through an increase in IGF-1 and Akt activity *(81,82)*. The inhibition of estrogen synthesis from androgen was also found to cause neurodegeneration in response to a usually nondamaging dose of kainic acid *(83)*. Although estrogen is believed to exert some of its neuroprotective actions through modulation of the innate immune response in the CNS, ovarectomized (OVX) female mice show an attenuated immune response to LPS delivered into their brain. The OVX mice also exhibit a more severe herpes simplex virus (HSV)-2 infection and associated neuronal damage than their sham-operated controls. These data indicate that estrogen is not necessarily anti-inflammatory but favors the transfer from innate to adaptive immunity. This may explain its neuroprotective role during HSV-2-induced encephalitis *(84)*.

The culminating evidence supporting the role of estrogen in neuroprotection has spurred research to investigate its potential as a therapeutic agent in women suffering from Alzheimer's disease as well as those with ischemic injury *(85)*.

Although not as well-documented, there is some evidence that testosterone can also play neuroprotective roles in the CNS. Testosterone was found to preserve hippocampal neurons following adrenalectomy in rats *(86)*. Additionally, castrated hamsters show poorer recovery from facial nerve transection than castrated hamsters receiving replacement testosterone therapy *(87)*. Clinical studies negatively correlated testosterone plasma levels with severity of illness in patients with Huntington's disease *(88)*. A possible mechanism of neuroprotection by testosterone was recently elucidated, whereby the activation of a MAPK/ERK pathway by the binding of the steroid to the androgen receptor spared neurons from β-amyloid toxicity in culture *(89)*. Testosterone also exerted its neuroprotective effects through the androgen-dependent extrahypothalamic actions of vasopressin, which has been posited as having anti-inflammatory properties *(90)*. Although the use of testosterone for the treatment of neurodegenerative diseases appears interesting, no significant in vivo studies have been performed.

The Brain's Cellular Cast: Puppets Under the Control of Soluble Neuroprotectors

It is certain that for neuroprotection to occur, signals must be sent from neurons in distress. In turn, the responding soluble effectors must bind to their receptors and/or act on gene responsive elements in neurons or other cells of the CNS to promote survival or prevent death (for an overview, *see* Fig. 6). At the heart of this response, the injured neuron must be able to communicate with its environment (and itself) to mobilize the CNS environment for its rescue. An interesting candidate for this signal by neurons is the fractalkine chemokine, which is tethered to the extracellular surface of neurons, and can be cleaved to diffuse in response to various stressors *(91)*. Receptors for fractalkine in the CNS are found mostly on the surface of microglia *(92)*, which are usually the first-line responders during brain injury. Upon acting on resident microglia and activating them, fractalkine can modulate their functions to prevent overactivation and neurotoxicity *(93)*. The controlled removal of apoptotic neurons is further promoted by the asymmetric distribution of the aminophospholipid phosphatidylserine to the extracellular layer of the neuronal cytoplasmic membrane, which regulates microglial–neuron communications and promotes phagocytosis of the neuron *(94)*. Heat shock protein (hsp) production in neurons is

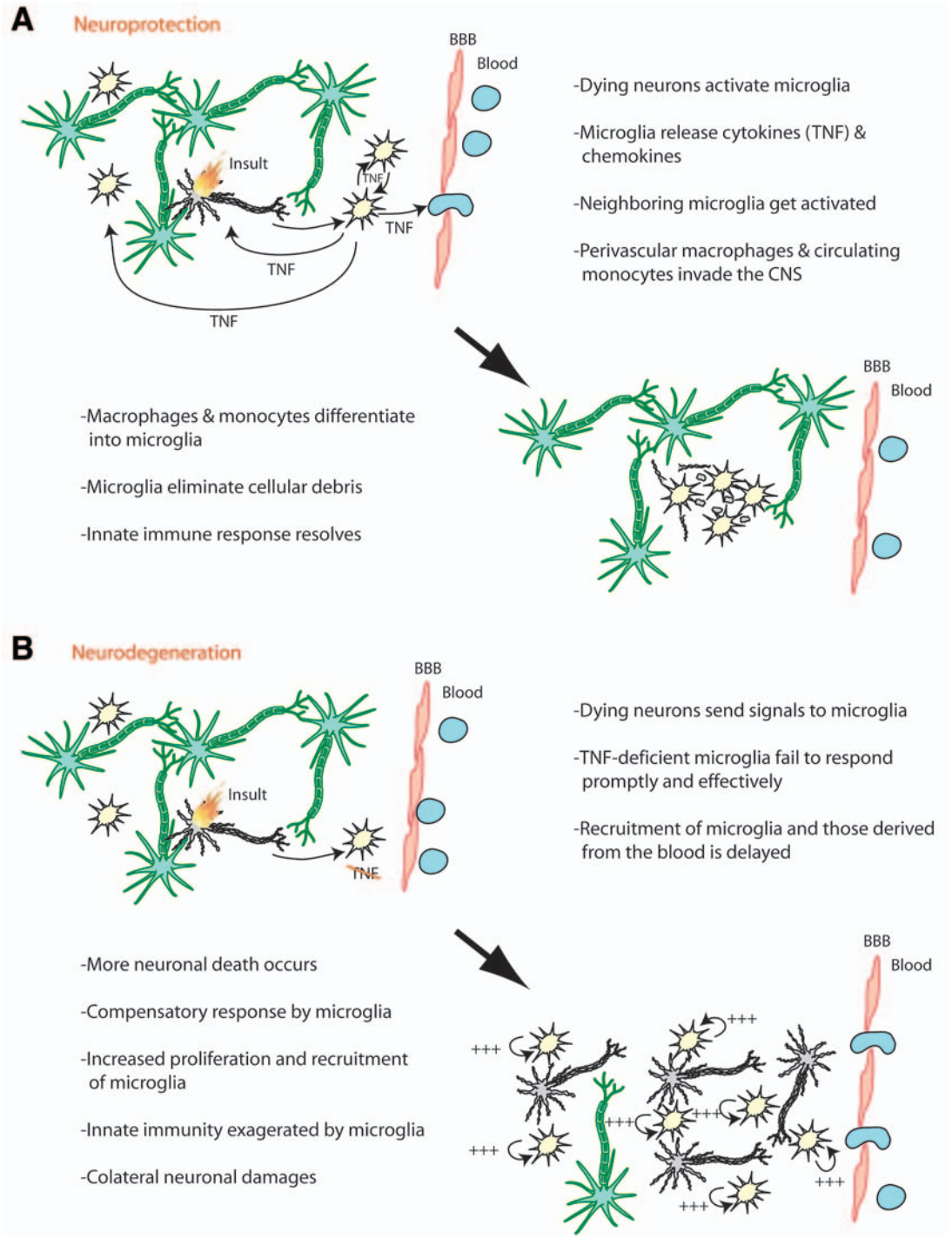


Fig. 6. The fate of neurons following injury is determined by the microglial response. In a scenario where microglia are able to respond promptly **(A)** they are able to receive signals from neurons and quickly signal other microglia and recruit blood monocytes to clear neuronal debris and resolve the CNS injury. In cases where microglia are not able to respond properly (e.g., when TNF-α is absent), **(B)** the attenuated early immune response prevents the quick clearance of the dying neurons, which leads to an exaggerated compensatory late response resulting from increased neuronal death. This results in collateral neuronal injuries and a perpetuated immune response at the site of the insult.

also increased when they are in distress and may provide the neurons with their own signals of self-preservation *(95)*. Furthermore, the release of hsps may trigger TLR signaling and activation in microglia, because some TLRs have been found to bind endogenous hsps *(96)*.

The early signals of distress from the CNS do not have to come from the neurons themselves but may also result from their demyelination. This involves the deterioration of the oligodendrocytes that support them and the release of their intracellular proteins. Upon the death of oligodendrocytes, neighboring microglia may phagocytose myelin and its associated proteins, such as myelin basic protein *(97)*. The clearing of oligodendritic debris is adaptive, but the possible presentation of myelin proteins by APCs to T-cells can occur, which has the dangerous potential of leading to autoimmune demyelinating conditions such as MS. Putting the inflammatory/immune response by microglia aside, the demyelination of neurons can stimulate the proliferation of OPCs, which can promote local remyelination of the axons *(98)*. However, the fate of the progenitors remains under debate, because they have the tendency to differentiate into astrocytes instead of oligodendrocytes, unless the proper treatment is applied *(99)*.

Discussion

This article has demonstrated how the CNS, through the coordinated effort of many soluble and cellular elements, is equipped with a multitude of endogenous strategies to protect itself from neuronal death and demyelination. As we have discussed, most of the neuroprotective strategies promote survival through the transient activation of microglia, neurotrophic factor upregulation, NF-κB signaling, and the activation of MAPK/ERK, Akt, and Bcl-2, Bcl-xL in different populations of cells in the CNS. In most cases, the fate of neurons is determined between the balance of pro- and anti-apoptotic signals. This is adaptive, preventing critically injured and nonfunctional neurons to uselessly survive. Furthermore, neuroprotective signals are extremely redundant, which permits compensation between signals to preserve a coherent response. However, neuroprotection needs to remain under tight reins because of the significant consequences that accompany a chronic or exaggerated inflammatory response in the brain. Such a loss of control over the CNS immune response can lead to an exacerbation of the damage and/or the establishment of an auto-immune state.

Even if the CNS is able to surmount most neurodegenerative challenges it faces, there are damages that are overwhelming because of their severity, their compromise to the BBB, or their slow subtle suppression that kept them under the neuroimmunological radar. When these cases occur, pharmacological and more invasive interventions (stem cells, epilepsy, etc.) have had marginal success at best. Most therapies have focused on the containment of the inflammatory response, which traditionally was believed only to impede neuroprotection and repair, unlike what we have outlined in this article. Furthermore, for most progressive neurodegenerative disorders such as ALS and Alzheimer's disease, when the neurodegeneration is finally detected, it is often too late to prevent the chain of events that leads to further neuronal death and/or demyelination.

Finally, because of the postmitotic state of neurons, repair strategies can only help to halt deterioration of these cells following injury. Strategies to improve cytokine and neurotrophic factor delivery by microglia or invading macrophages could represent a possible solution to this problem *(100)* and improve the immune response in the brain rather than hindering it. Better diagnostic tools that will delineate early warning or susceptibility to neurodegenerative disease will also provide us with more efficient approaches to prevent CNS abnormalities (protein aggregates, unusual neuronal firing, reaction to myelin antigens by T-cells, etc.) from getting out of hand and precipitating uncontrollable CNS cell death *(101)*.

Acknowledgments

N. P. Turrin is supported by a fellowship from the Multiple Sclerosis of Canada, and S. Rivest currently holds a research chair from the Canadian Institute for Health Research. Because of the great amount of research currently done on neuroprotection, the authors would like to apologize for the possible omission of key work or findings that were not cited in the current review.

References

1. Dirnagl U., Iadecola C., and Moskowitz M. A. (1999) Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci*. **22**, 391–397.
2. Lo E. H., Dalkara T., and Moskowitz M. A. (2003) Mechanisms, challenges and opportunities in stroke. *Nat. Rev. Neurosci*. **4**, 399–415.
3. Gasic G. P. and Nicotera P. (2003) To die or to sleep, perhaps to dream. *Toxicol. Lett*. **139**, 221–227.
4. Nixon R. A. (2006) Autophagy in neurodegenerative disease: friend, foe or turncoat? *Trends Neurosci*. **9,** 528–535.
5. Akwa Y., Allain H., Bentue-Ferrer D., et al. (2005) Neuroprotection and neurodegenerative diseases: from biology to clinical practice. *Alzheimer Dis. Assoc. Disord*. **19**, 226–239.
6. Ekshyyan O. and Aw T. Y. (2004) Apoptosis: a key in neurodegenerative disorders. *Curr. Neurovasc. Res*. **1**, 355–371.
7. Hawkins R. A., O'Kane R. L., Simpson I. A., and Vina J. R. (2006) Structure of the blood–brain barrier and its role in the transport of amino acids. *J. Nutr*. **136**, 218S–226S.
8. Hawkins B. T. and Davis T. P. (2005) The blood–brain barrier/neurovascular unit in health and disease. *Pharmacol. Rev*. **57**, 173–185.
9. Turrin N. P. and Rivest S. (2004) Unraveling the molecular details involved in the intimate link between the immune and neuroendocrine systems. *Exp. Biol. Med. (Maywood)*. **229**, 996–1006.
10. Buller K. M. (2001) Role of circumventricular organs in pro-inflammatory cytokine-induced activation of the hypothalamic-pituitary-adrenal axis. *Clin. Exp. Pharmacol. Physiol*. **28**, 581–589.
11. Kielian T. (2006) Toll-like receptors in central nervous system glial inflammation and homeostasis. *J. Neurosci. Res*. **83**, 711–730.
12. Anderson K. V. (2000) Toll signaling pathways in the innate immune response. *Curr. Opin. Immunol*. **12**, 13–19.
13. Kaur C., Hao A. J., Wu C. H., and Ling E. A. (2001) Origin of microglia. *Microsc. Res. Tech*. **54**, 2–9.
14. Simard A. R. and Rivest S. (2004). Bone marrow stem cells have the ability to populate the entire central nervous system into fully differentiated parenchymal microglia. *FASEB J*. **18**, 998–1000.
15. Massengale M., Wagers A. J., Vogel H., and Weissman I. L. (2005) Hematopoietic cells maintain hematopoietic fates upon entering the brain. *J. Exp. Med*. **201**, 1579–1589.
16. Priller J., Flugel A., Wehner T., et al. (2001) Targeting gene-modified hematopoietic cells to the central nervous system: use of green fluorescent protein uncovers microglial engraftment. *Nat. Med*. **7**, 1356–1361.
17. Simard A. R., Soulet D., Gowing G., Julien J. P., and Rivest S. (2006) Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. *Neuron* **49**, 489–502.
18. Dong Y. and Benveniste E. N. (2001) Immune function of astrocytes. *Glia* **36**, 180–190.
19. Lubetzki C., Williams A., and Stankoff B. (2005) Promoting repair in multiple sclerosis: problems and prospects. *Curr. Opin. Neurol*. **18**, 237–244.
20. Engelhardt B. and Ransohoff R. M. (2005) The ins and outs of T-lymphocyte trafficking to the CNS: anatomical sites and molecular mechanisms. *Trends Immunol*. **26**, 485–495.
21. Olson J. K., Ludovic Croxford J., and Miller S. D. (2004) Innate and adaptive immune requirements for induction of autoimmune demyelinating disease by molecular mimicry. *Mol. Immunol*. **40**, 1103–1108.
22. Vilhardt F. (2005) Microglia: phagocyte and glia cell. *Int. J. Biochem. Cell. Biol*. **37**, 17–21.
23. Nadeau S. and Rivest S. (2003) Glucocorticoids play a fundamental role in protecting the brain during innate immune response. *J. Neurosci*. **23**, 5536–5544.
24. Rudehill S., Muhallab S., Wennersten A., et al. (2006) Autoreactive antibodies against neurons and basal lamina found in serum following experimental brain contusion in rats. *Acta. Neurochir. (Wien)*. **148**, 199–205.
25. Ciric B., Howe C. L., Paz Soldan M., et al. (2003) Human monoclonal IgM antibody pro-

motes CNS myelin repair independent of Fc function. *Brain Pathol*. **13**, 608–616.
26. Glezer I., Lapointe A., and Rivest S. (2006) Innate immunity triggers oligodendrocyte progenitor reactivity and confines damages to brain injuries. *FASEB J*. **20**, 750–752.
27. Turrin N. P. and Rivest S. (2006) Tumor necrosis factor alpha but not interleukin 1 beta mediates neuroprotection in response to acute nitric oxide excitotoxicity. *J. Neurosci*. **26**, 143–151.
28. Marchetti L., Klein M., Schlett K., Pfizenmaier K., and Eisel U. L. (2004). Tumor necrosis factor (TNF)-mediated neuroprotection against glutamate-induced excitotoxicity is enhanced by N-methyl-D-aspartate receptor activation. Essential role of a TNF receptor 2-mediated phosphatidylinositol 3-kinase-dependent NF-kappa B pathway. *J. Biol. Chem*. **279**, 32,869–32,881.
29. Arnett H. A., Mason J., Marino M., Suzuki K., Matsushima G. K., and Ting J. P. (2001) TNF alpha promotes proliferation of oligodendrocyte progenitors and remyelination. *Nat. Neurosci*. **4**, 1116–1122.
30. Blais V. and Rivest S. (2004) Effects of TNF-alpha and IFN-gamma on nitric oxide-induced neurotoxicity in the mouse brain. *J. Immunol*. **172**, 7043–7052.
31. Fontaine V., Mohand-Said S., Hanoteau N., Fuchs C., Pfizenmaier K., and Eisel U. (2002) Neurodegenerative and neuroprotective effects of tumor Necrosis factor (TNF). in retinal ischemia: opposite roles of TNF receptor 1 and TNF receptor 2. *J. Neurosci*. **22**, RC216.
32. Kennedy S. G., Kandel E. S., Cross T. K., and Hay N. (1999) Akt/Protein kinase B inhibits cell death by preventing the release of cytochrome c from mitochondria. *Mol. Cell Biol*. **19**, 5800–5810.
33. Guo Z., Iyun T., Fu W., Zhang P., and Mattson M. P. (2004) Bone marrow transplantation reveals roles for brain macrophage/microglia TNF signaling and nitric oxide production in excitotoxic neuronal death. *Neuromolecular Med*. **5**, 219–234.
34. Dhandapani K. M., Wade F. M., Wakade C., Mahesh V. B., and Brann D. W. (2005) Neuroprotection by stem cell factor in rat cortical neurons involves AKT and NFkappaB. *J. Neurochem*. **95**, 9–19.
35. Bartesaghi S., Marinovich M., Corsini E., Galli C. L., and Viviani B. (2005) Erythropoietin: a novel neuroprotective cytokine. *Neurotoxicology*. **26**, 923–928.
36. Sakanaka M., Wen T. C., Matsuda S., et al. (1998) In vivo evidence that erythropoietin protects neurons from ischemic damage. *Proc. Natl. Acad. Sci. USA*. **95**, 4635–4640.
37. Brines M. L., Ghezzi P., Keenan S., et al. (2000) Erythropoietin crosses the blood-brain barrier to protect against experimental brain injury. *Proc. Natl. Acad. Sci. USA*. **97**, 10,526–10,531.
38. Celik M., Gokmen N., Erbayraktar S., et al. (2002) Erythropoietin prevents motor neuron apoptosis and neurologic disability in experimental spinal cord ischemic injury. *Proc. Natl. Acad. Sci. USA*. **99**, 2258–2263.
39. Ehrenreich H., Hasselblatt M., Dembowski C., et al. (2002) Erythropoietin therapy for acute stroke is both safe and beneficial. *Mol. Med*. **8**, 495–505.
40. Siren A. L., Fratelli M., Brines M., et al. (2001) Erythropoietin prevents neuronal apoptosis after cerebral ischemia and metabolic stress. *Proc. Natl. Acad. Sci. USA*. **98**, 4044–4049.
41. Chong Z. Z., Lin S. H., Kang J. Q., and Maiese K. (2003) Erythropoietin prevents early and late neuronal demise through modulation of Akt1 and induction of caspase 1, 3, and 8. *J. Neurosci. Res*. **71**, 659–669.
42. Ghezzi P. and Brines M. (2004) Erythropoietin as an antiapoptotic, tissue-protective cytokine. *Cell Death Differ*. **11(Suppl 1)**, S37–S44.
43. Viviani B., Bartesaghi S., Corsini E., et al. (2005) Erythropoietin protects primary hippocampal neurons increasing the expression of brain-derived neurotrophic factor. *J. Neurochem*. **93**, 412–421.
44. Mitrasinovic O. M., Grattan A., Robinson C. C., et al. (2005) Microglia overexpressing the macrophage colony-stimulating factor receptor are neuroprotective in a microglial-hippocampal organotypic coculture system. *J. Neurosci*. **25**, 4442–4451.
45. Berezovskaya O., Maysinger D., and Fedoroff S. (1996) Colony stimulating factor-1 potentiates neuronal survival in cerebral cortex ischemic lesion. *Acta Neuropathol. (Berl)*. **92**, 479–486.
46. Vincent V. A., Robinson C. C., Simsek D., and Murphy G. M. (2002) Macrophage colony stimulating factor prevents NMDA-induced neuronal death in hippocampal organotypic cultures. *J. Neurochem*. **82**, 1388–1397.
47. Ahmad A. S., Ahmad M., de Brum-Fernandes A. J., and Dore S. (2005) Prostaglandin EP4 receptor agonist protects against acute neurotoxicity. *Brain Res*. **1066**, 71–77.

48. Liu D., Wu L., Breyer R., Mattson M. P., and Andreasson K. (2005) Neuroprotection by the PGE2 EP2 receptor in permanent focal cerebral ischemia. *Ann. Neurol*. **57**, 758–761.
49. McCullough L., Wu L., Haughey N., et al. (2004) Neuroprotective function of the PGE2 EP2 receptor in cerebral ischemia. *J. Neurosci*. **24**, 257–268.
50. Bilak M., Wu L., Wang Q., et al. (2004) PGE2 receptors rescue motor neurons in a model of amyotrophic lateral sclerosis. *Ann. Neurol*. **56**, 240–248.
51. Ahmad A. S., Saleem S., Ahmad M., and Dore S. (2006) Prostaglandin EP1 receptor contributes to excitotoxicity and focal ischemic brain damage. *Toxicol. Sci*. **89**, 265–270.
52. Liang X., Wang Q., Hand T., et al. (2005) Deletion of the prostaglandin E2 EP2 receptor reduces oxidative damage and amyloid burden in a model of Alzheimer's disease. *J. Neurosci*. **25**, 10,180–10,187.
53. Toyomoto M., Ohta M., Okumura K., et al. (2004) Prostaglandins are powerful inducers of NGF and BDNF production in mouse astrocyte cultures. *FEBS Lett*. **562**, 211–215.
54. Bazan N. G., Marcheselli V. L., and Cole-Edwards K. (2005) Brain response to injury and neurodegeneration: endogenous neuroprotective signaling. *Ann. NY Acad. Sci*. **1053**, 137–147.
55. Filbin M. T. (2006) How inflammation promotes regeneration. *Nat. Neurosci*. **9**, 715–717.
56. Yin Y., Henzl M. T., Lorber B., et al. (2006) Oncomodulin is a macrophage-derived signal for axon regeneration in retinal ganglion cells. *Nat. Neurosci*. **9**, 843–852.
57. Kalb R. (2005) The protean actions of neurotrophins and their receptors on the life and death of neurons. *Trends Neurosci*. **28**, 5–11.
58. Royo N. C., Conte V., Saatman K. E., et al. (2006) Hippocampal vulnerability following traumatic brain injury: a potential role for neurotrophin-4/5 in pyramidal cell neuroprotection. *Eur. J. Neurosci*. **23**, 1089–1102.
59. Leeds P., Leng Y., Chalecka-Franaszek E., and Chuang D. M. (2005) Neurotrophins protect against cytosine arabinoside-induced apoptosis of immature rat cerebellar neurons. *Neurochem. Int*. **46**, 61–72.
60. McCollum A. T. and Estus S. (2004) NGF acts via p75 low-affinity neurotrophin receptor and calpain inhibition to reduce UV neurotoxicity. *J. Neurosci. Res*. **77**, 552–564.
61. Sharma H. S. (2003) Neurotrophic factors attenuate microvascular permeability disturbances and axonal injury following trauma to the rat spinal cord. *Acta Neurochir. Suppl*. **86**, 383–388.
62. Majdan M. and Miller F. D. (1999) Neuronal life and death decisions functional antagonism between the Trk and p75 neurotrophin receptors. *Int. J. Dev. Neurosci*. **17**, 153–161.
63. Longo F. M. and Massa S. M. (2004) Neurotrophin-based strategies for neuroprotection. *J. Alzheimers Dis*. **6**, S13–S17.
64. Levy D. E. and Lee C. K. (2002) What does Stat3 do? *J. Clin. Invest*. **109**, 1143–1148.
65. Naumann T., Schnell O., Zhi Q., et al. (2003) Endogenous ciliary neurotrophic factor protects GABAergic, but not cholinergic, septohippocampal neurons following fimbria-fornix transection. *Brain Pathol*. **13**, 309–321.
66. Vanderlocht J., Hellings N., Hendriks J. J., et al. (2006) Leukemia inhibitory factor is produced by myelin-reactive T cells from multiple sclerosis patients and protects against tumor necrosis factor-alpha-induced oligodendrocyte apoptosis. *J. Neurosci. Res*. **83**, 763–774.
67. Zang da W. and Cheema S. S. (2003) Leukemia inhibitory factor promotes recovery of locomotor function following spinal cord injury in the mouse. *J. Neurotrauma*. **20**, 1215–1222.
68. Pizzi M., Sarnico I., Boroni F., et al. (2004) Prevention of neuron and oligodendrocyte degeneration by interleukin-6 (IL-6) and IL-6 receptor/IL-6 fusion protein in organotypic hippocampal slices. *Mol. Cell Neurosci*. **25**, 301–311.
69. Adcock I. M., Ito K., and Barnes P. J. (2004) Glucocorticoids: effects on gene transcription. *Proc. Am. Thorac. Soc*. **1**, 247–254.
70. Glezer I. and Rivest S. (2004) Glucocorticoids: protectors of the brain during innate immune responses. *Neuroscientist* **10**, 538–552.
71. Sloka J. S. and Stefanelli M. (2005) The mechanism of action of methylprednisolone in the treatment of multiple sclerosis. *Mult. Scler*. **11**, 425–432.
72. Mihai C. and Jubelt B. (2005) Post-infectious encephalomyelitis. *Curr. Neurol. Neurosci. Rep*. **5**, 440–445.
73. Kurkowska-Jastrzebska I., Litwin T., Joniec I., et al. (2004) Dexamethasone protects against dopaminergic neurons damage in a mouse model of Parkinson's disease. *Int. Immunopharmacol*. **4**, 1307–1318.
74. Hall E. D. and Springer J. E. (2004) Neuroprotection and acute spinal cord injury: a reappraisal. *NeuroRx* **1**, 80–100.
75. Qian T., Guo X., Levi A. D., Vanni S., Shebert R. T., and Sipski M. L. (2005) High-dose methyl-

prednisolone may cause myopathy in acute spinal cord injury patients. *Spinal Cord*. **43**, 199–203.
76. Alderson P. and Roberts I. (2005) Corticosteroids for acute traumatic brain injury. *Cochrane Database Syst. Rev*. CD000196.
77. Amantea D., Russo R., Bagetta G., and Corasaniti M. T. (2005) From clinical evidence to molecular mechanisms underlying neuroprotection afforded by estrogens. *Pharmacol. Res*. **52**, 119–132.
78. Alonso de Lecinana M. and Egido J. A. (2006) Estrogens as neuroprotectants against ischemic stroke. *Cerebrovasc. Dis*. **21(Suppl 2)**, 48–53.
79. Won C. K., Kim M. O., and Koh P. O. (2006) Estrogen modulates Bcl-2 family proteins in ischemic brain injury. *J. Vet. Med. Sci*. **68**, 277–280.
80. Alexaki V. I., Charalampopoulos I., Kampa M., et al. (2006) Activation of membrane estrogen receptors induce pro-survival kinases. *J. Steroid Biochem. Mol. Biol*. **98**, 97–110.
81. D'Astous M., Mickley K. R., Dluzen D. E., and Di Paolo T. (2005) Differential protective properties of estradiol and tamoxifen against methamphetamine-induced nigrostriatal dopaminergic toxicity in mice. *Neuroendocrinology* **82**, 111–120.
82. D'Astous M., Mendez P., Morissette M., Garcia-Segura L. M., and Di Paolo T. (2006) Implication of the phosphatidylinositol-3 kinase/protein kinase B signaling pathway in the neuroprotective effect of estradiol in the striatum of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mice. *Mol. Pharmacol*. **69**, 1492–1498.
83. Veiga S., Azcoitia I., and Garcia-Segura L. M. (2005) Extragonadal synthesis of estradiol is protective against kainic acid excitotoxic damage to the hippocampus. *Neuroreport* **16**, 1599–1603.
84. Soucy G., Boivin G., Labrie F., and Rivest S. (2005) Estradiol is required for a proper immune response to bacterial and viral pathogens in the female brain. *J. Immunol*. **174**, 6391–6398.
85. Behl C. (2006) The search for novel avenues for the therapy and prevention of Alzheimer's disease. *Drug News Perspect*. **19**, 5–12.
86. Frye C. A. and McCormick C. M. (2000) Androgens are neuroprotective in the dentate gyrus of adrenalectomized female rats. *Stress* **3**, 185–194.
87. Coers S., Tanzer L., and Jones K. J. (2002) Testosterone treatment attenuates the effects of facial nerve transection on glial fibrillary acidic protein (GFAP) levels in the hamster facial motor nucleus. *Metab. Brain Dis*. **17**, 55–63.
88. Markianos M., Panas M., Kalfakis N., and Vassilopoulos D. (2005) Plasma testosterone in male patients with Huntington's disease: relations to severity of illness and dementia. *Ann. Neurol*. **57**, 520–525.
89. Nguyen T. V., Yao M., and Pike C. J. (2005) Androgens activate mitogen-activated protein kinase signaling: role in neuroprotection. *J. Neurochem*. **94**, 1639–1651.
90. Dantzer R., Bluthe R. M., and Kelley K. W. (1991) Androgen-dependent vasopressinergic neurotransmission attenuates interleukin-1-induced sickness behavior. *Brain Res*. **557**, 115–120.
91. Chapman G. A., Moores K., Harrison D., Campbell C. A., Stewart B. R., and Strijbos P. J. (2000) Fractalkine cleavage from neuronal membranes represents an acute event in the inflammatory response to excitotoxic brain damage. *J. Neurosci*. **20**, RC87.
92. Verge G. M., Milligan E. D., Maier S. F., Watkins L. R., Naeve G. S., and Foster A. C. (2004) Fractalkine (CX3CL1) and fractalkine receptor (CX3CR1) distribution in spinal cord and dorsal root ganglia under basal and neuropathic pain conditions. *Eur. J. Neurosci*. **20**, 1150–1160.
93. Mizuno T., Kawanokuchi J., Numata K., and Suzumura A. (2003) Production and neuroprotective functions of fractalkine in the central nervous system. *Brain Res*. **979**, 65–70.
94. De Simone R., Ajmone-Cat M. A., and Minghetti L. (2004) Atypical antiinflammatory activation of microglia induced by apoptotic neurons: possible role of phosphatidylserine-phosphatidylserine receptor interaction. *Mol. Neurobiol*. **29**, 197–212.
95. Latchman D. S. (2004) Protective effect of heat shock proteins in the nervous system. *Curr. Neurovasc. Res*. **1**, 21–27.
96. Vabulas R. M., Wagner H., and Schild H. (2002) Heat shock proteins as ligands of toll-like receptors. *Curr. Top. Microbiol. Immunol*. **270**, 169–184.
97. Li Y., Field P. M., and Raisman G. (1999) Death of oligodendrocytes and microglial phagocytosis of myelin precede immigration of Schwann cells into the spinal cord. *J. Neurocytol*. **28**, 417–427.
98. Di Bello I. C., Dawson M. R., Levine J. M., and Reynolds R. (1999) Generation of oligodendroglial progenitors in acute inflammatory demyelinating lesions of the rat brain stem is associated with demyelination rather than inflammation. *J. Neurocytol*. **28**, 365–381.

99. Tatsumi K., Haga S., Matsuyoshi H., Inoue M., Manabe T., Makinodan M., and Wanaka A. (2005) Characterization of cells with proliferative activity after a brain injury. *Neurochem. Int*. **46**, 381–389.
100. Lesniak M. S., Kelleher E., Pardoll D., and Cui Y. (2005) Targeted gene therapy to antigen-presenting cells in the central nervous system using hematopoietic stem cells. *Neurol. Res*. **27**, 820–826.
101. Werner P. (2003) Knowledge about symptoms of Alzheimer's disease: correlates and relationship to help-seeking behavior. *Int. J. Geriatr. Psychiatry* **18**, 1029–1036.