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T cell-microglial dialogue in Parkinson's disease and amyotrophic lateral sclerosis: are we listening?

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Neuroinflammation is a pathological hallmark in Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS), and is characterized by activated microglia and infiltrating T cells at sites of neuronal injury. In PD and ALS, neurons do not die alone; neuronal injury is non-cell-autonomous and depends on a well-orchestrated dialogue in which neuronally secreted misfolded proteins activate microglia and initiate a self-propagating cycle of neurotoxicity. Diverse populations and phenotypes of CD4+ T cells crosstalk with microglia, and depending on their activation status, influence this dialogue and promote neuroprotection or neurotoxicity. A greater understanding of the T cell population that mediates these effects, as well as the molecular signals involved should provide targets for neuroprotective immunomodulation to treat these devastating neurodegenerative diseases.

Introduction

The central nervous system (CNS) traditionally has been considered immunologically privileged, but over the years there has been a dramatic reevaluation of this tenet. Current data suggest that the CNS is immunocompetent and actively interacts with the peripheral immune system. Neuroinflammation is now recognized to be a prominent feature of many classic neurodegenerative disorders and can mediate either neuroprotection or neurotoxicity [1]. However, controversy still exists and this highlights the importance of a more thorough understanding of the toxic versus protective effects of the immune responses in the CNS.

Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS) are neurodegenerative diseases that present predominantly as sporadic, and to a lesser extent, inheritable disorders. The etiology of the sporadic forms is unknown, but our understanding of disease pathogenesis has gained considerable momentum following the discovery of familial and toxin-induced forms of disease, which has led to the development of animal models. In ALS, the discovery of mutations in Cu^{2+}/Zn^{2+} superoxide dismutase (mSOD1) as the most common cause of familial ALS (fALS) has led to the development of mSOD1 transgenic animal models and an exponential increase in our understanding of disease pathogenesis [2,3]. In PD, the discovery that

neurotoxins can produce the symptoms and signs of parkinsonism [4] has prompted the development of animal models that have elucidated the molecular pathways of dopaminergic (DA) cell injury. Information gleaned from both ALS and PD tissues and animal models has suggested that mitochondrial dysfunction, increased reactive oxygen species (ROS), misfolded and aggregated proteins, and dysfunction of the ubiquitin proteosome pathway are key events that provoke neurodegeneration [3,5]. Yet, in both PD and ALS, a detailed molecular understanding of what causes neuronal death is lacking. Elegant experiments in the mSOD1 mouse have suggested that neurons do not die alone, but rather the process is non-cell-autonomous [6] and depends on the active participation of non-neuronal cells such as microglia, astrocytes and T cells. In PD and ALS, microglial activation and T-cell infiltration are significant pathological hallmarks at sites of significant neuronal injury, yet such neuroinflammation has been considered the consequence and not the cause of neuronal injury. The present review challenges this dogma and suggests that both innate and adaptive immune systems respond to, as well as contribute to the pathology and tissue destruction.

PD

PD is a chronic CNS progressive disorder that is characterized by tremor, rigidity and bradykinesia (slowness of movement), caused by compromise of the nigrostriatal pathway as well as other DA and non-DA neurons [5]. The pathological hallmarks of PD are loss of nigrostriatal DA neurons, especially those projecting to the putamen, and the presence of Lewy bodies, which are intraneuronal proteinaceous cytoplasmic inclusions [7]. The degree of DA terminal loss in the striatum appears to be more pronounced than the loss of substantia nigra (SN) DA neurons, which suggests that DA terminals are the primary target, which results in a "dying back" process [5]. Degeneration of the ventral midbrain DA neurons is accompanied by activation of microglia (the innate immune cells of the CNS) and infiltration of T lymphocytes (cells of the adaptive immune system).

Microglia

Microglia are the resident immunocompetent cells within the CNS, and display functional plasticity during activation, which involves changes in cell number, morphology, surface

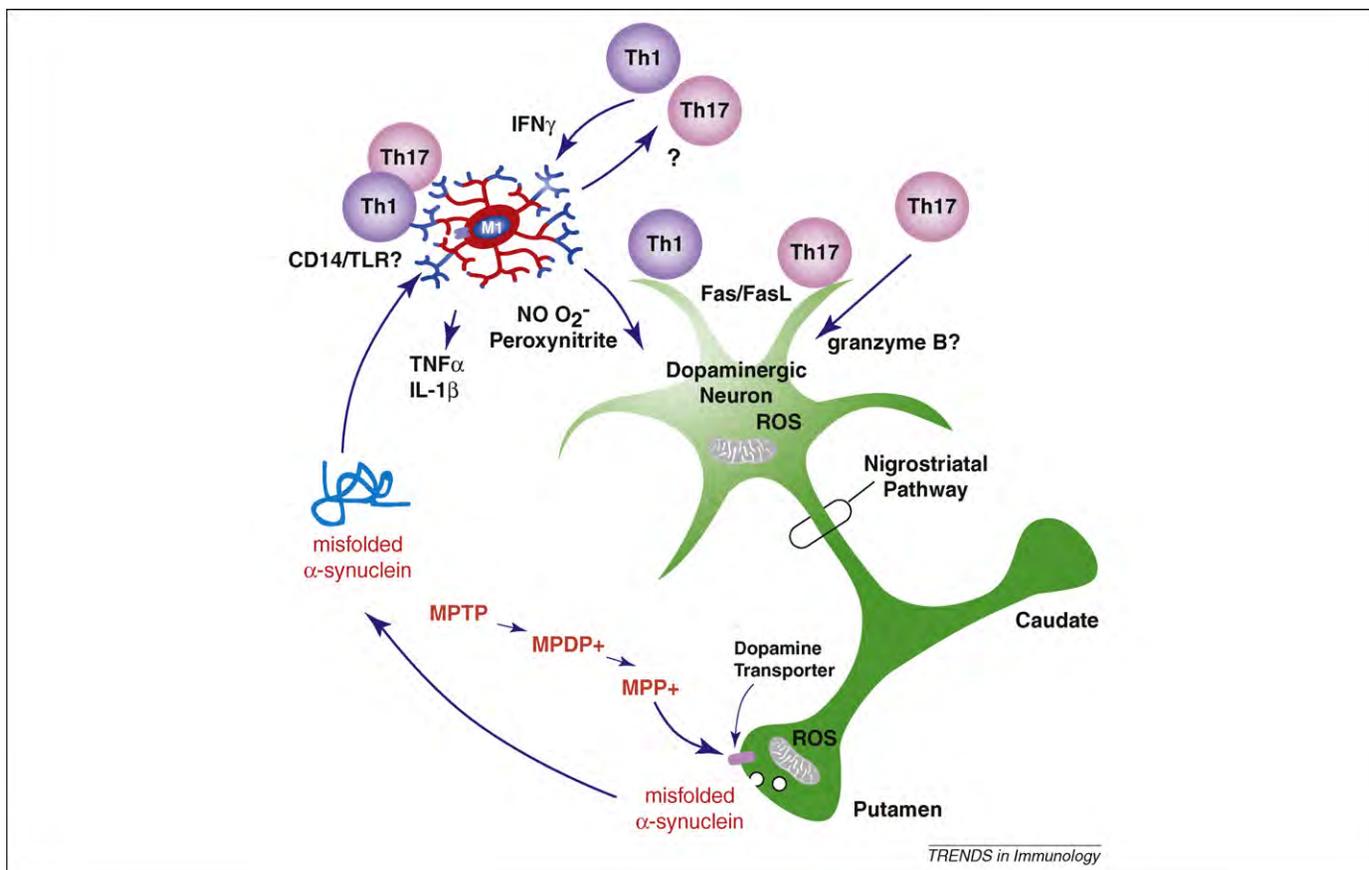Corresponding author: Appel, S.H. (sappel@tmhs.org).

Figure 1. Communication between a neuron and the immune system in a chemically induced model of PD. The neurotoxin MPTP is first converted to MPDP$^+$ and then to MPP$^+$ by non-DA cells, for example, glia or serotonergic neurons [5]. MPP$^+$ is concentrated in dopaminergic cells via presynaptic dopamine transporters. In the presence of MPP$^+$, ROS produced by mitochondria in the SN DA neurons induce misfolded α-syn, which is released subsequently in vesicles at presynaptic terminals. The misfolded α-syn in turn activates microglia, possibly via a receptor, transforming microglia into a neurotoxic M1 microglial phenotype. Misfolded α-syn is released from presynaptic terminals in the striatum and activates microglia in proximity to DA cell somas in the SN. It is likely that α-syn is also released from DA cell bodies and activates local microglia, as well as being released from presynaptic DA terminals and activating adjacent microglia. Potential receptors for misfolded α-syn on microglia include CD14/TLRs, and the scavenger receptors. The M1 microglia influence Th1 and perhaps Th17 T cells, which in turn maintain the M1 microglial phenotype via production of IFN-γ. The M1 microglia release NO and $O_2^{\bullet-}$ form the highly reactive and toxic peroxynitrite. These ROS compound the injury to DA neurons. M1 microglia also release pro-inflammatory cytokines, such as TNF-α and IL-1β, and amplify the toxic inflammation. T cells can also directly injure DA neurons by signaling through the Fas/FasL system.

receptor expression, and production of growth factors and cytokines [8]. The changes reflect altered activation states induced by signals that arise from injured neurons and surrounding glia (Figure 1). As with macrophages, microglia can exhibit a classically activated M1 phenotype or an alternatively activated M2 phenotype [9,10] (Box 1). Classically activated M1 microglia secrete increased pro-inflammatory cytokines [tumor necrosis factor (TNF)-α and interleukin (IL)-1β] and potent ROS [superoxide radicals ($O_2^{\bullet-}$) and nitric oxide (NO)], and reduced neurotrophic factors. At least three different M2 phenotypes have been identified based upon their gene expression profile, and cover a continuum of functional states [11]. The M2 alternatively activated (M2a) phenotype, induced by IL-4 or IL-13 with increased expression of the characterisitc proteins arginase-1, FIZZ1 (found in inflammatory zone 1), and chitinase 3-like protein (Ym1), is distinguished from two others that include the M2b phenotype, induced by immune complexes and Toll-like receptor (TLR) agonists, and the M2-deactivated (M2c) phenotype that is induced by IL-10 or transforming growth factor (TGF)-β with increased expression of chemokine receptor 2 (CCR2) and scavenger receptors [12]. M2 microglia can enhance neurotrophic factor release, such as insulin-like growth factor 1 (IGF-1), reduce

pro-inflammatory cytokines, and assist in inflammation resolution.

The CNS also has important neuroprotective mechanisms that can inhibit directly peripheral and central immune responses, but these have been less well studied. One potential mechanism involves CD200, which is highly expressed on neurons and can downregulate immune activity through binding of the microglial receptor CD200R. CD200 and CD200R belong to the immunoglobulin superfamily. CD200 transmits an immunoregulatory signal through the CD200R to regulate microglial activation [13].

In PD, activated microglia are prominent, surround DA neurons, and exhibit a classically activated M1 phenotype [14,15]. Increased lipid peroxidation, as well as carbonyl- and nitrotyrosine-modified proteins, is also observed in CNS nigral tissue [16]. Activated microglia that surround DA neurons are noted in postmortem tissues of human subjects that have previously developed symptoms and signs of parkinsonism, following exposure to the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [4] (see Box 2 for further details). As a result of the relationship between neuronal CD200 and microglial CD200R, it has been proposed that CD200–CD200R signaling is correlated

closely to microglial activation in PD, and has the potential of modulating the pathogenesis and progression of PD [17].

MPTP mouse model of PD

The discovery that MPTP induces parkinsonian features in humans has led to the development of MPTP-induced parkinsonian animal models for defining the potential role of innate and adaptive immune systems in the pathophysiology of DA neuronal injury. In MPTP-injected mice, M1 microglia are associated topographically and temporally with DA neurodegenerative changes (Figure 1). These activated microglia release increased levels of NO and $O_2^{\bullet-}$ through the induction of NO synthase and NADPH oxidase (NOX) [18,19]. MPTP-treated mutant mice defective in NO synthase or NADPH oxidase secrete less NO or $O_2^{\bullet-}$ and exhibit less neuronal loss, with reduced levels of nitrotyrosine- and carbonyl-modified proteins than their MPTP-treated wild-type (WT) littermates [18,19]. Such data suggest that, in the MPTP model, activated microglia injure DA neurons, possibly by peroxynitrite generated from $O_2^{\bullet-}$ and NO. Further support for this suggestion has come from *in vitro* studies that have demonstrated that activated microglia can induce DA neuron death directly [20].

Chemokines are chemotactic cytokines that play a key role in chemotaxis and activation of immune cells such as microglia. In the CNS, these chemokines are released by glial cells during an inflammatory response, and in addition to their chemotactic functions, they can play a role in neuromodulation and cell survival [21]. In the MPTP model of murine PD, monocyte chemoattractant protein-1 (CCL2) is upregulated in the striatum and the ventral midbrain. Astrocytes are the predominant source of CCL2 in the striatum and the SN, and DA neurons in the SN constitutively express CCL2 [22]. MPTP treatment results in decreased CCL2 expression in the surviving DA neurons. However, deletion of the genes that encode CCL2 and its major receptor CCR2, does not affect MPTP-induced striatal dopamine depletion. These results suggest that recruitment of resident microglia or peripheral monocytes following MPTP treatment does not protect against striatal dopamine loss in this model of PD.

In the MPTP model, the initiation of neuronal injury has been attributed to MPP$^+$ mediated blockade of mitochondrial complex I (see Box 2). In mouse striatal slices and isolated DA neurons, the blockade of complex I increases the production of ROS, and enhances oxidation and nitration of DA neuronal proteins [23–25]. A prominent neuronal protein that undergoes oxidation is α-synuclein (α-syn). This protein is enriched in synaptic vesicles at presynaptic terminals of DA neurons, and accumulates as misfolded and aggregated protein in familial PD patients with mutations in the α-syn (*SNCA*) gene, or when the WT α-syn protein is overexpressed [26,27]. These misfolded aggregates, including conformationally altered α-syn by dopamine treatment, are degraded poorly by chaperone-mediated autophagy, and block degradation of other substrates [28]. Inhibition of autophagy further enhances misfolding of α-syn and contributes to the selective degeneration of PD DA neurons.

A key question in the MPTP model is whether the mitochondrial dysfunction, increased ROS, and misfolded α-syn within DA neurons are sufficient to cause DA neuron death [29]. Recent evidence suggests that misfolded α-syn-induced cytotoxicity is non-cell-autonomous and requires the participation of non-neuronal cells such as microglia. Normal monomeric and aggregated forms of α-syn are secreted from DA neurons, and in turn, the aggregated α-syn activates microglia and upregulates NADPH oxidase and production of ROS [29–31]. Inhibitors of microglial production of NO and $O_2^{\bullet-}$ attenuate *in vitro* DA neurotoxicity [32]. Expression of the human α-syn protein in

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Box 2. Metabolism of MPTP

In 1982, young intravenous drug users developed a rapidly progressive parkinsonian syndrome caused by the use of a street preparation of 1-methyl-4-phenyl-4-propionoxypiperidine (MPPP), an analog of the narcotic meperidine (Demerol). MPTP, a neurotoxic contaminant inadvertently produced during the illicit synthesis of MPPP, produces an irreversible and severe syndrome characterized by all of the features of PD, including tremor, rigidity, slowness of movement, postural instability, and freezing. The highly lipophilic MPTP crosses the blood–brain barrier within minutes, where it is oxidized to 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP$^+$) by monoamine oxidase B in glia and serotonergic neurons, the only cells that produce this enzyme. MPDP$^+$ is then converted to the active toxin MPP$^+$, probably by spontaneous oxidation, and released by an unknown mechanism into the extracellular space. MPP$^+$ is a high-affinity substrate for the dopamine transporter, as well as for noradrenaline and serotonin transporters, on neurons. Inside neurons, MPP$^+$ can bind to the vesicular monoamine transporter-2, which translocates MPP$^+$ into synaptosomal vesicles; it can be concentrated within the mitochondria by a mechanism that relies on the mitochondrial transmembrane potential; or it can remain in the cytosol to interact with cytosolic enzymes. Vesicular sequestration of MPP$^+$ appears to protect cells from neurodegeneration by sequestering the toxin and preventing it from accessing the mitochondria. However, once inside the mitochondria, MPP$^+$ impairs oxidative phosphorylation by inhibiting complex I of the mitochondrial electron transport chain, which leads to decreases in tissue ATP content. MPTP administration leads to the accumulation and nitration of α-syn in the cytosol of DA neurons. Adapted from reference [5].

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transgenic mice results in accumulation of insoluble α-syn aggregates in nigral neurons, and renders DA neurons more vulnerable to lipopolysaccharide (LPS)-induced microglia-mediated neurotoxicity [32]. Thus, DA neurons exposed to MPP$^+$ have increased accumulation and secretion of misfolded and aggregated α-syn, thereby activating microglia to release free radicals and pro-inflammatory cytokines, and initiating a self-propagating cycle of DA neuronal injury. The increased release of misfolded and aggregated α-syn causes further oxidation and release of DA neuronal α-syn, and further amplification of DA neurodegeneration.

Role of T cells in PD patients and animal models
The potential role of T cells in PD pathogenesis has been investigated less extensively. T cells were originally documented to be present in the SN of PD patients [14]. Subsequent reports have confirmed the presence of CD4$^+$ and CD8$^+$ T cells in the SN of patients with PD [33]. In the murine MPTP model, T cells can enhance cytotoxicity because severe combined immunodeficiency (SCID) mice, which lack T cells, are relatively resistant to MPTP-induced degeneration of SN neurons [34]. Similarly, MPTP-induced DA cell death is attenuated markedly in the absence of mature T cells in two other different immunodeficient mouse strains ($Rag1^{-/-}$ and $Tcrb^{-/-}$ mice) [33]. Attenuation of MPTP-induced DA cell death is noted in mice that lack CD4$^+$ T cells, as well as in $Rag1^{-/-}$ mice reconstituted with Fas ligand (FasL)-deficient splenocytes; i.e., splenocytes with impaired cell-mediated killing ability [33]. MPTP-induced DA cell death is not attenuated in mice that lack CD8$^+$ T cells [33]. Furthermore, transfer of whole T-cell populations from mice immunized with nitrated α-syn (i.e. misfolded) accelerates MPTP-induced DA cell loss

[34]. Collectively, these data indicate that the deleterious activity of MPTP involves infiltrating CD4$^+$ T cells with signals mediated through the Fas/FasL pathway.

At present, the specific CD4$^+$ T-cell subpopulation(s) (e.g. Th1, Th17) that modulates cytotoxicity in MPTP-treated mice is unknown. Whether microglia dictate the specific T-cell phenotype or T cells dictate the specific microglial phenotype (i.e. M1 *vs.* M2) in MPTP mice is also unknown. Activated microglia and the cytokine milieu that they generate might promote T-cell differentiation into various functional lineages. M1 cells promote, whereas M2 cells decrease, proliferation and function of CD4$^+$ Th1 cells [35]. Conversely, T cells dictate microglial anti- or pro-inflammatory phenotypes. CD4$^+$ Th1 cells secrete IFN-γ, which in turn activates M1 microglia (or promotes their differentiation) and provokes loss of DA neurons [36] Th17 cells also might contribute to neurotoxicity, possibly through the secretion of IL-17 or release of granzyme B, a cytolytic enzyme (Figure 1) [37]. Activated T cells also can injure neurons directly through cell-contact-dependent mechanisms that involve FasL, lymphocyte function-associated antigen 1, and CD40 but not major histocompatibility class (MHC) class I; i.e. independent of peptide presentation by neurons [38].

T cells also can be neuroprotective, as demonstrated in MPTP-injected mice in which the passive transfer of T cells obtained from mice immunized with the immunomodulatory drug glatiramer acetate (GA) is neuroprotective [39–41] (Figure 2). The injection of GA-derived T cells attenuates the MPTP-induced SN cell loss, possibly by increasing secretion of the anti-inflammatory cytokines IL-4 and IL-10, as well as TGF-β, and decreasing microglial release of pro-inflammatory factors. GA is used classically in the treatment of multiple sclerosis (MS), in which it has been documented to induce Th2-polarized immune responses that could mediate neuroprotection through the release of IL-4, which suppresses the production and release of free radicals from activated microglia [42,43]. An alternative neuroprotective effect of GA might be to modulate the differentiation and survival of Th17 cells. This may operate by altering STAT3 (signal transducer and activator of transcription 3) phosphorylation and subsequent expression of the RORγ (retinoic acid receptor-related orphan receptor gamma) transcription factor that is necessary for the differentiation and survival of Th17 cells [44]. However, it has not been established definitively whether the neuroprotective functions of GA in the MPTP models derive from enhanced Th2 or CD4$^+$CD25$^+$ regulatory T (Treg) cell functions, or reduced Th17 or Th1 functions.

Although initial investigations attribute most GA activity to a preferential Th2 response, recent reports indicate that GA treatment can also exert immunomodulatory activity on monocytes/macrophages and dendritic cells [45]. *In vitro*, GA induces the transcription and production of secreted IL-1 receptor antagonist (sIL-1Ra), an inhibitor of the pro-inflammatory mediator IL-1β, in human monocytes, and is considered an important regulator of inflammation and the overall immune response mediated by IL-1. Of relevance to chronic inflammation in PD, GA has been shown to diminish strongly the expression of IL-1β and enhance the production of sIL-1Ra from
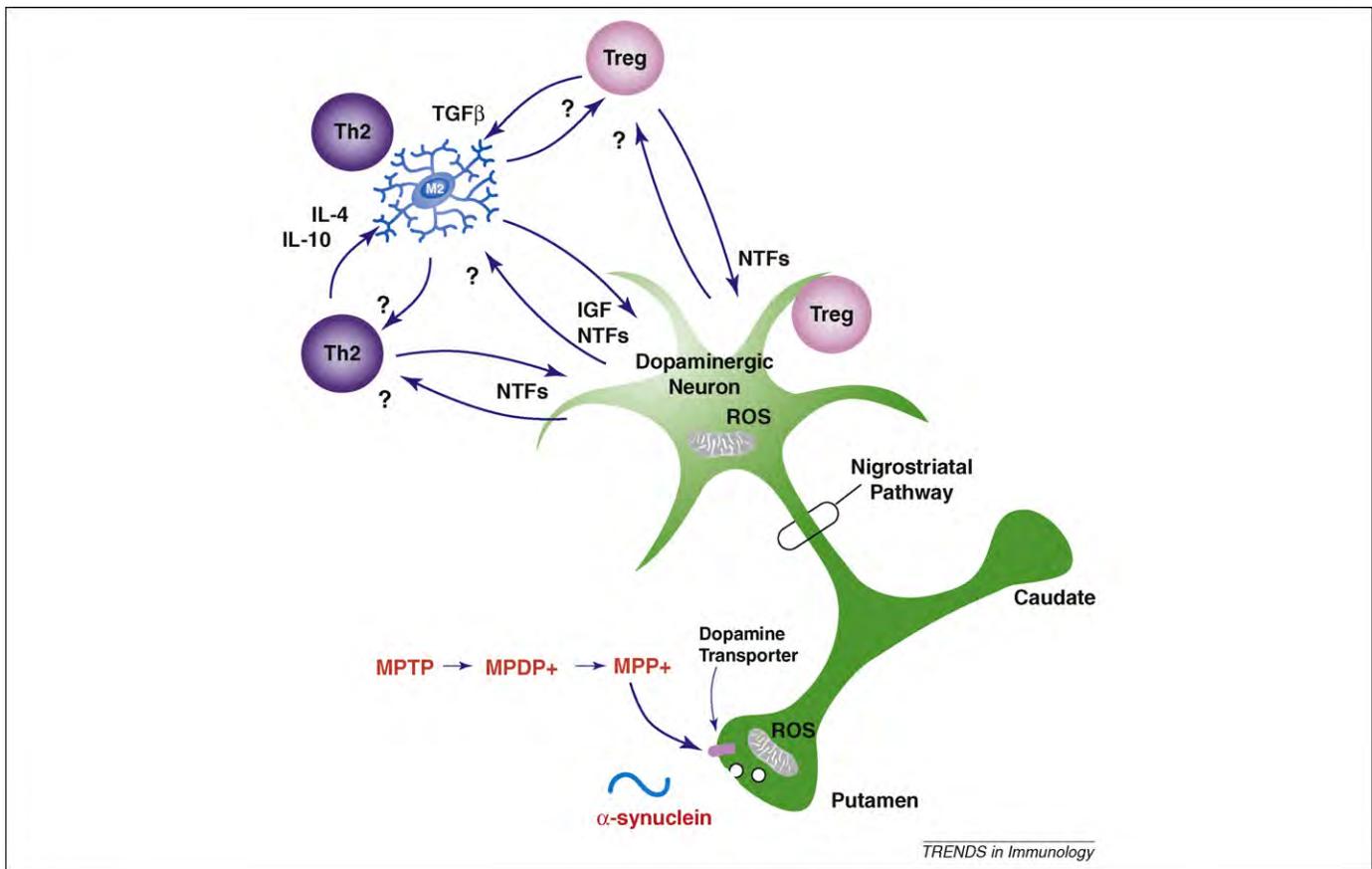
Figure 2. An immunologically protective model following adaptive transfer of T cells in MPTP-induced PD. Th2 and Treg cells maintain a neuroprotective M2 microglial phenotype through the release of IL-4, IL-10 and TGF-β. Treg cells suppress microglial synthesis and release of ROS induced by misfolded α-syn. Th2 and Treg cells might also protect neurons directly by cell-contact-dependent mechanisms or by releasing BDNF and other neurotrophic factors. M2 microglia release IGF-1 and other neurotrophic factors, which protect DA neurons in the SN. M2 microglia might also positively influence Th2 and Treg cells. Injured neurons might themselves induce M2 microglia and attract Th2 and Tregs cells via release of chemokines.

monocytes in a model of T-cell induced monocyte activation. These results suggest that a potential mechanism for GA neuroprotection includes enhancing the levels of circulating sIL-1Ra, thereby directly affecting monocytes and biasing the response towards a less inflammatory profile.

Neuroprotection can be mediated by Treg cells. In the PD model, increased protection against MPTP-induced DA cell death has been reported following adoptive transfer of activated Treg cells, whereas CD4$^+$CD25$^-$ effector T (Teff) cells do not provide protection from MPTP-induced DA cell loss [46]. *In vitro,* Treg cells suppress microglial synthesis and release of ROS induced by misfolded α-syn, whereas Teff cells exacerbate microglial release of ROS [47]. The functional state of CD4$^+$ T cells is highly dependent on their cytokine milieu, and it is not clear in MPTP-injected mice how long Treg cells remain functional in a milieu of M1-activated microglia that secrete pro-inflammatory cytokines and ROS. Furthermore, it is uncertain in the MPTP model whether Treg cells mediate neuroprotection by suppressing cytotoxic T cells such as Th1 or Th17, by converting pro-inflammatory M1 microglial phenotypes into anti-inflammatory M2 microglia, or by direct neurotrophic factor support. Nevertheless, it is clear that, in models of PD, microglia and T cells are important participants in mediating cytotoxicity and neuroprotection. Therefore, a greater understanding of the initiating signals

and the specific milieu that dictates innate and adaptive immune responses might provide opportunities for novel therapeutic approaches in patients with PD.

ALS
ALS, commonly known as Lou Gehrig's disease, is an adult-onset, rapidly progressive disorder that selectively destroys upper and lower motor neurons, which results in various degrees of weakness and atrophy of limb musculature, spasticity, dysarthria (slurred speech), and dysphagia (difficulty swallowing), and death, typically within 4–6 years [3,48]. At present, therapy is mainly symptomatic, and fails to halt disease progression. Denervation at the neuromuscular junction is observed prior to motor neuron loss in the spinal cord, which suggests that motor neuron pathology begins at the distal axon and proceeds in a "dying back" process, similar to that observed in PD [5,49]. As in patients with PD, neuroinflammation is a prominent pathological feature of sporadic ALS (sALS), which is characterized by activated microglia and infiltrating T cells at sites of spinal cord motor neuron injury [50–52]. In addition, increased monocytes/macrophages, dendritic cells, and CCL2 are present in ALS spinal cord tissue [45]. Spinal cord tissue from ALS patients also has increased mRNA levels for specific dendritic cell markers that are associated with shortened lifespans after diagnosis, which suggests that dendritic cells contribute to motor
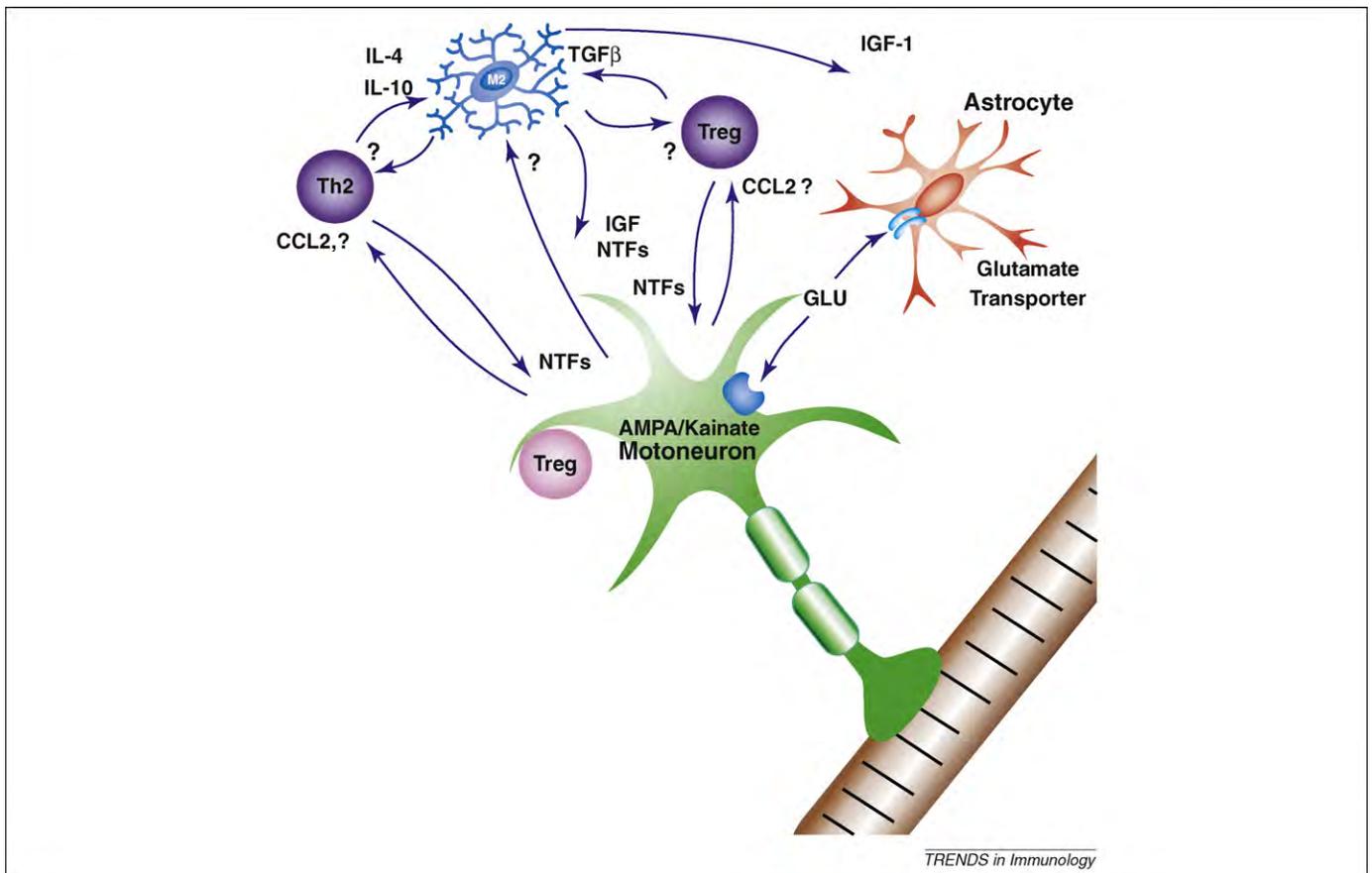
Figure 3. The innate and adaptive immune systems help maintain motor neuron homeostasis in an early phase of ALS, as well as in the facial axotomy model. Initially, injured neurons attract and maintain neuroprotective M2 microglia by an unknown signal and attract Th2 and Treg cells by chemokines, possibly CCL2. The first response to neuronal injury might be neuroprotective, and then with sustained neuronal stress, there is a transformation to a cytotoxic response. The M2 microglial phenotype could be the CNS initial default state in PD and ALS. M2 microglia secrete IGF-1 and other neurotrophic factors, which also enhance the neuroprotective properties of astrocytes; the astrocytic glutamate transporters are functional and actively remove glutamate. Th2 and Treg cells secrete neurotrophic factors, which directly protect motor neurons. IL-4, IL-10, and TGF-β secreted by Th2 and Treg cells help maintain M2 microglia, and M2 microglia also influence Th2 and Treg cells. Treg cells have been shown to differentiate macrophages directly toward the M2 state. M2 microglia can induce suppressive Treg cells.

neuron injury [52]. The increased CCL2 is of considerable importance because it is known to enhance the trafficking of T cells into the CNS [53]. Depending on their phenotype and activation status, T cells may cross-talk with neurons and microglia, and either damage or protect neurons from stressful stimuli [54]. However, it is unclear whether microglia and/or T cells are neuroprotective, cytotoxic, or possibly both, in ALS. As in PD, animal models of ALS have provided clues to the possible role of the immune system in motor neuron injury and cell death (Figure 3).

mSOD1 animal model of ALS
SOD1 is a ubiquitously expressed cytosolic enzyme that converts highly reactive and toxic $O_2^{\bullet-}$ molecules to H_2O_2; peroxidases then detoxify H_2O_2 to form water. SOD1 mutations cause fALS and transgenic animals overexpressing different SOD1 mutations develop a chronic progressive motor neuron disease similar to human fALS [2,55–57] (Box 3). T cells, activated microglia, immunoglobulins, and dendritic cells are present in the spinal cords of mSOD1 mice [58–60]. Activated microglia are observed at an early age prior to signs of disease onset. Numerous studies of mSOD1 mice also suggest immune activation of microglia with increased expression of inducible nitric oxide
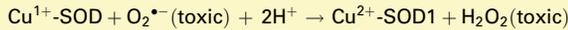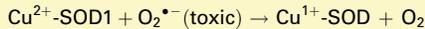
synthase, the catalytic subunit of the phagocyte NADPH oxidase (NOX2), IL-10, IL-6, TNF-α and CCL2 [60–65]. Although it is not clear if these responses are protective or injurious, there is clear evidence of an early immune response in the transgenic mSOD1 animal models of fALS (Figure 4).

Role of microglia in mSOD1 models
Glutamate is the most abundant excitatory neurotransmitter in the CNS and amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainite receptors are the major glutamate receptors on motor neurons. Glutamate is normally non-toxic, but excessive levels of glutamate or modification of AMPA/kainite receptors result in increased influx of calcium and neuronal death. Activated microglia can induce motor neuron cell death *in vitro* by releasing free radicals and increasing the susceptibility of the motor neuron AMPA/kainate receptor to the toxic effects of glutamate [66]. Furthermore, microglia that express mSOD1 are more readily activated and induce more motor neuron cell death than WT microglia [67]. To demonstrate that microglia contribute to motor neuron injury *in vivo*, we have used PU.1$^{-/-}$ transgenic mice that are unable to develop myeloid and lymphoid cells. Following bone marrow transplantation (BMT), the entire CNS parenchyma is

Box 3. A brief history of SOD1

SOD1 binds copper and zinc ions and is one of three isozymes responsible for removing $O_2^{\bullet-}$ in the body. The enzymatic action of SOD1, a 153-amino-acid ubiquitously expressed homodimer, is to convert $O_2^{\bullet-}$, which is produced primarily by errors of oxidative phosphorylation in mitochondria, to water and H_2O_2. Catalysis by SOD1 is mediated in two asymmetric steps by an essential copper atom, which is alternately reduced and oxidized by superoxide:

$$Cu^{2+}\text{-SOD1} + O_2^{\bullet-}(toxic) \rightarrow Cu^{1+}\text{-SOD} + O_2$$

$$Cu^{1+}\text{-SOD} + O_2^{\bullet-}(toxic) + 2H^+ \rightarrow Cu^{2+}\text{-SOD1} + H_2O_2(toxic)$$

$$H_2O_2(toxic) \xrightarrow{\text{Glutathione peroxidase, catalase}} H_2O$$

Cu^{1+}-SOD1 may also use H_2O_2 as a substrate to produce the reactive hydroxyl radical:

$$Cu^{1+}\text{-SOD} + H_2O_2(toxic) \rightarrow Cu^{1+}\text{-SOD-OH} + OH^-$$

$$Cu^{1+}\text{-SOD-OH} + OH^- \rightarrow Cu^{1+}\text{-SOD} + {}^{\bullet}OH(toxic)$$
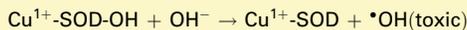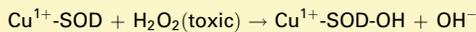
In 1993, the discovery of missense mutations in the SOD1 gene in fALS directed research efforts toward identifying the mechanisms by which mSOD1 confers selective motor neuron toxicity. Currently, more than 130 different mutations distributed throughout SOD1 polypeptide have been linked to fALS. A distinct manganese superoxide dismutase (SOD2) is resident in mitochondria, yet only mutations in the soluble cytoplasmic SOD1 enzyme cause fALS. An initial hypothesis suggested that mSOD1 has reduced SOD1 enzymatic activity, thus promoting accumulation of toxic $O_2^{\bullet-}$. However, this hypothesis was not validated because several mSOD1 retain some, if not all, enzymatic activity. Furthermore, deletion of WT SOD1 does not cause an ALS phenotype. Thus, mSOD1 is now thought to confer a toxic ''gain-of-function'' independent of enzymatic activity, possibly caused by misfolding of the mSOD1 protein. This toxic ''gain-of-function'' underlies the many current hypotheses for the pathobiology of fALS; none of which is solely responsible for the pathogenesis of disease. What is clear is that mSOD1 does cause oxidative damage, accumulation of intracellular aggregates, endoplasmic reticular stress, mitochondrial dysfunction, defects in axonal transport, growth factor deficiency, astroglial cell pathology, and glutamate excitotoxicity. To this extensive list, neuroinflammation, which is characterized by activated microglia and infiltrating T cells, should be added because it is clearly involved in the pathophysiology of this devastating neurodegenerative disease. Adapted from reference [94].

colonized with microglia of donor-derived origin [68]. In this model, mSOD1 microglia do not initiate disease when all other cells in the mouse, including motor neurons, do not express mSOD1. However, we have demonstrated that replacing mSOD1 microglia with WT microglia slows motor neuron loss, prolongs disease duration (from disease onset until death), and extends survival of mSOD1 mice [68]. Reduced expression of mSOD1 in microglia or macrophages also slows disease progression and prolongs survival [69]. These data suggest that WT microglia or microglia that express less mSOD1 exhibit a different phenotype and promote neuroprotection in ALS mice. Therefore, it is likely that microglia have both neuroprotective and neurotoxic functions, depending on the different states of microglial activation that are elicited by local factors and stimuli.

The fact that mSOD1 expression in both microglia and motor neurons enhances cytotoxicity raises the possibility that proteins or other signals released from motor neurons activate microglia and contribute to neurotoxicity, just as misfolded and aggregated α-syn released from stressed DA neurons activates microglia and initiates a self-propagating amplification of DA cell injury. In the ALS model, the signal could be mSOD1 itself, because mSOD1 protein bound to chromogranin (a secretory granule protein) is secreted from neural cells and triggers microgliosis and neuronal death in mixed spinal cord cultures [70]. Although mSOD1 protein does not directly injure motor neurons, it can activate microglia and trigger release of ROS and induce motor neuron injury and cell death through a CD14/TLR pathway [71]. Of possible relevance to sALS, WT SOD1 acquires binding and toxic properties of mSOD1 through oxidative damage, and might activate microglia and induce motor neuron death in spinal cord cultures [72]. Furthermore, an altered SOD1 species has been detected within the spinal cord of sALS patients that must have originated from misfolded WT SOD1, possibly unifying a shared pathophysiological pathway between sALS and fALS [73].

Role of T cells in the mSOD1 animal model

To determine whether T cells modulate the microglial–motor neuron dialogue in mSOD1 mice, we have bred mSOD1 mice that lack functional T cells or CD4$^+$ T cells [60]. The unexpected result was that motor neuron disease is accelerated, which suggests that CD4$^+$ T cells are neuroprotective, and not cytotoxic as noted in the MPTP experiments. Furthermore, the early, relatively slow phase of disease progression is eliminated in the absence of functional CD4$^+$ T cells, accompanied by increased proinflammatory and cytotoxic factors, decreased anti-inflammatory and neurotrophic factors, and decreased survival. BMT reconstitutes mice with CD4$^+$ T cells, restores the early, slowly progressive phase of disease, prolongs survival, and suppresses cytotoxicity. The immunomodulatory effects of the CD4$^+$ neuroprotective T cells appear to enhance an M2-activated microglial phenotype with increased anti-inflammatory factors and neurotrophic factors [74]. Transgenic mice with the same SOD1 mutation, but bred with different T-cell-deficient ($Tcr3^{-/-}$) mice, have confirmed that T cells play an endogenous neuroprotective role [75]. Reconstitution of mSOD1 mice with *ex vivo* expanded Treg or Teff cells from WT donor mice delays the loss of motor function and enhancesd survival [76]. Our own preliminary data are in accord with these results and suggest that CD4$^+$ T cells are neuroprotective in mSOD1×RAG2$^{-/-}$ mice during the stable phase, and that CD4$^+$ T cells obtained from mSOD1 mice are even more protective than CD4$^+$ T cells from WT mice [74].

These studies suggest that Treg or Th2 cells, in conjunction with M2 microglia, contribute to neuroprotection in ALS models [74]. If Treg cells are responsible, it could be attributable to secretion of TGF-β and IL-10, or suppression of cytotoxic-T-cell function and downregulation of proinflammatory cytokine production. Treg cells have been shown to differentiate macrophages directly toward the M2 state, upregulate the mannose and scavenger receptors, and increase phagocytic capacity [77]. Thymus-derived natural Treg cells can decrease M2 cell HLA-DR class II
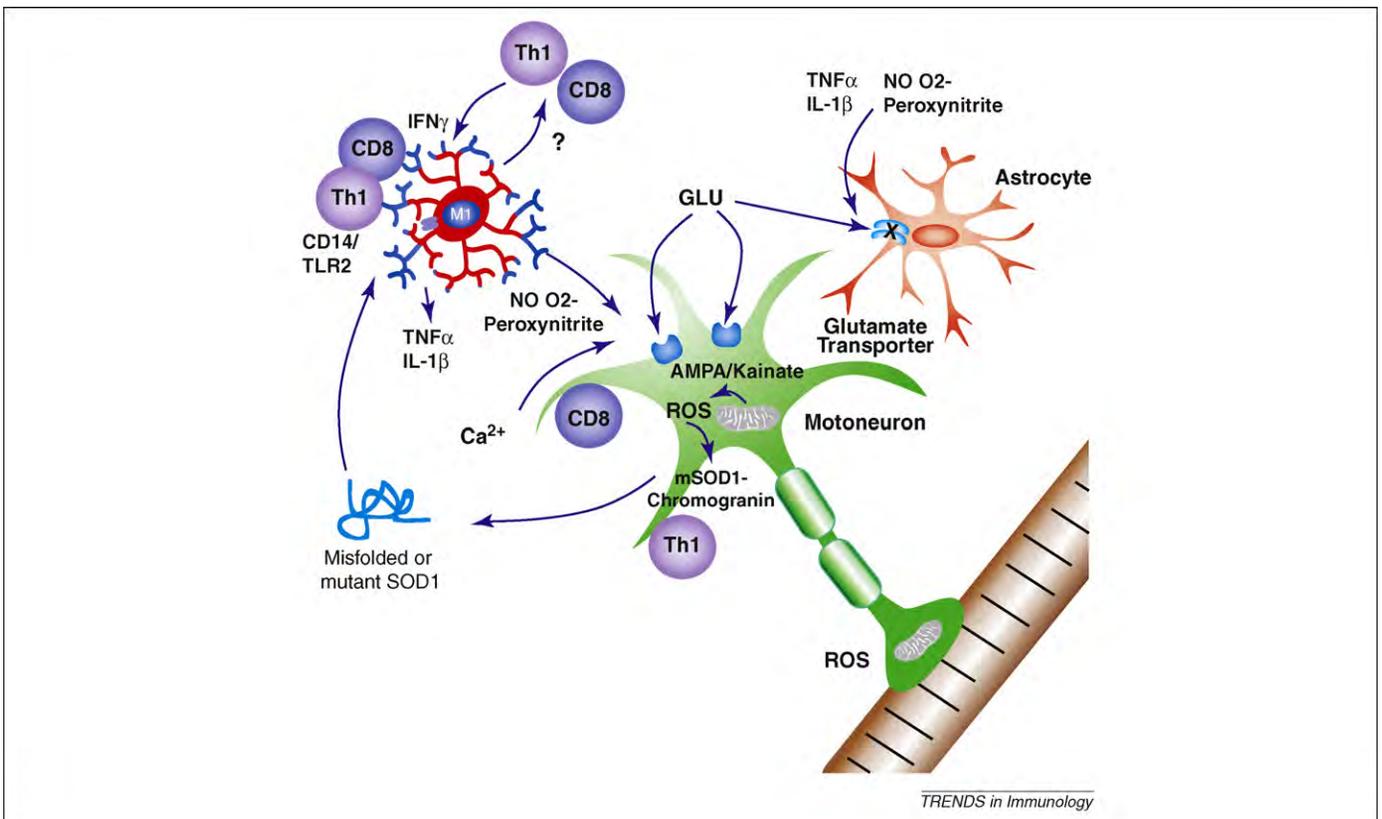
Figure 4. An immunological shift from protection to neurotoxicity accelerates disease in ALS. Initial presentation of mSOD1 or oxidized and misfolded WT SOD1 to T cells might occur in the periphery. Later in disease, as the concentration of extracellular mutant or misfolded SOD1 accumulates, this now rogue protein activates the CD14/TLRs on microglia and transforms them from a protective M2 phenotype to a neurotoxic M1 phenotype. Microglia might also re-present the rogue protein to T cells (Th1 and CD8). *In vitro*, M1 microglia release the pro-inflammatory TNF-α and IL-1β cytokines and NO and O$_2^{\bullet-}$, which may form the highly reactive and toxic peroxynitrite. These ROS alter the motor neuron AMPA/kainate channel, which results in increased glutamate susceptibility and subsequent Ca^{2+} influx cytotoxicity. Extracellular glutamate concentrations may increase because of reduced numbers and ability of the glutamate transporters expressed by astrocytes to remove glutamate, which results in further glutamate toxicity. The pro-inflammatory cytokines and ROS can also render the astroglial glutamate transporters ineffective, which further compounds the Ca^{2+}-induced neurotoxicity. The M1 microglia also influence Th1 and CD8 T cells, which in turn maintain M1 microglial phenotype through the release of IFN-γ, and can also exert direct toxic effects on motor neurons.

expression and reduce their capacity to produce pro-inflammatory mediators in response to LPS (a TLR4 ligand) [77]. M2 cells can induce Treg cells with a strong suppressive function, and Treg cell function has been shown to be controlled tightly by the maturation state of dendritic cells [78]. Maintenance of M2 macrophage activation also requires CD4$^+$ Th2 cells [79] Furthermore, microglia that encounter anti-inflammatory Th2 cells, or IL-4, support neuronal survival via production of IGF-1 [43,80]. Other murine studies suggest that neurodegeneration is augmented by the presence of Treg and Teff cells, but either alone is neuroprotective [81,82]. In the later, rapidly progressing phases of the disease, microglia have an M1 phenotype that expresses increased pro-inflammatory cytokines and reactive-oxygen-generating enzymes [74]. At present, it is unclear whether, at these later stages, the neuroprotective CD4$^+$ T cells (e.g. Treg or Th2) have been transformed into more cytotoxic CD4$^+$ T cells (e.g. Th1 or Th17 cells); our preliminary evidence suggests this is not the case. Neurons themselves cannot be excluded as indirect mediators of disease, since they might have a crucial role in governing the T-cell response and CNS inflammation [83,84].

Neuroprotective T cells in the facial axotomy model
Studies with facial nerve axotomy have provided insight into the neuroprotective effects of T cells. Following facial

axotomy, i.e. severing of facial axons, microglia that surround the facial nerve cell body are activated and T cells are recruited subsequently to the motor neuron soma in the brain stem [85]. Specific evidence for T cells being neuroprotective is derived from studies in which immunodeficient mice that lack functional T and B cells have significantly reduced facial motor neuron survival [86]. Reconstitution of these mice with WT whole splenocytes restores facial motor neuron survival to WT levels. However, T cells have been reported also to have divergent effects in intact and immunodeficient mice [87]. Nevertheless, differentiated CD4$^+$ T cells that secrete brain-derived neurotrophic factor (BDNF) rescue facial motor neurons after axotomy [88–91]. CD4$^+$ T-cell neuroprotection depends on resident microglia and bone-marrow-derived antigen-presenting cells [92]. The activation of microglia, which causes them to function as antigen-presenting cells, and hence to express MHC class II proteins, is correlated clearly with a better outcome following CNS injury [93].

Although the neuroprotective signals released from motor neurons undergoing axotomy have not been defined clearly, the overall response is neuroprotective because the final consequence of axotomy in the adult rodent is the functional restoration of the facial motor neuron, its axon, and its synaptic connections. However, important

physiological information remains unknown, especially the first signals produced by injured motor neurons that initiate a neuroprotective response. Therefore, characterization of the cell-contact-dependent and -independent signaling involved in the T cell–microglia, neuron–microglia, and T cell–neuron dialogues are likely to be of significant therapeutic value [84].

Concluding remarks

Neuroinflammation, which is characterized by activated microglia and infiltrating T cells, is a prominent pathological feature in the neurodegenerative diseases PD and ALS. The evidence presented in this review clearly suggests that the immune system is involved in the pathophysiology of these two devastating neurodegenerative diseases. Experimental models suggest that activated microglia and CD4$^+$ T cells contribute significantly to disease progression but probably not to disease initiation. Data from rodent axotomy models and early stages of disease in mSOD1 mice indicate that the first response to injury, provided by the surrounding predominantly M2 microglia and infiltrating CD4$^+$ T cells, is neuroprotective. However, in murine models of PD and later stages of disease in mSOD1 mice, this neuroprotective response is not initiated or does not persist, and is transformed into a cytotoxic response, possibly because of continued neuronal stress and signaling. It is unknown at present why and how neuroprotection is transformed into cytotoxicity. In the MPTP model of PD and the mSOD1 model of ALS, misfolded and aggregated proteins, either α-syn in PD models and mutant or oxidized SOD1 in ALS models, are secreted from neurons, which promotes pro-inflammatory M1 microglia and cytotoxic T cells, and amplifies neuronal injury. In sporadic human PD and ALS, that is, in the absence of known mutations, it is unknown what initiates disease onset, but a similar set of temporal and mechanistic events could transform neuroprotective microglia and T cells into cytotoxic cells, thereby accelerating disease progression. Thus, the dialogue among microglia, T cells and neurons suggests that the immune response is not merely a consequence of injury, but actively influences and significantly contributes to the balance between neuroprotection and neurotoxicity. It is clear that neurodegeneration is non-cell autonomous, with the innate and adaptive immune systems contributing to neuronal viability and disease progression. A greater understanding of what dictates the presence of cytotoxic or neuroprotective immunomodulation and how to limit cytotoxicity and enhance neuroprotection would help identify appropriate targets for immune-based therapy in neurodegenerative diseases.

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