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REVIEW

Protective and Toxic Neuroinflammation in Amyotrophic Lateral Sclerosis

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Abstract Amyotrophic lateral sclerosis (ALS) is a clinically heterogeneous disorder characterized by loss of motor neurons, resulting in paralysis and death. Multiple mechanisms of motor neuron injury have been implicated based upon the more than 20 different genetic causes of familial ALS. These inherited mutations compromise diverse motor neuron pathways leading to cell-autonomous injury. In the ALS transgenic mouse models, however, motor neurons do not die alone. Cell death is noncell-autonomous dependent upon a well orchestrated dialogue between motor neurons and surrounding glia and adaptive immune cells. The pathogenesis of ALS consists of 2 stages: an early neuroprotective stage and a later neurotoxic stage. During early phases of disease progression, the immune system is protective with glia and T cells, especially M2 macrophages/microglia, and T helper 2 cells and regulatory T cells, providing anti-inflammatory factors that sustain motor neuron viability. As the disease progresses and motor neuron injury accelerates, a second rapidly progressing phase develops, characterized by M1 macrophages/microglia, and proinflammatory T cells. In rapidly progressing ALS patients, as in transgenic mice, neuroprotective regulatory T cells are significantly decreased and neurotoxicity predominates. Our own therapeutic efforts are focused on modulating these neuroinflammatory pathways. This review will focus on the cellular players involved in neuroinflammation in ALS and current therapeutic strategies to enhance neuroprotection and suppress neurotoxicity with the goal of arresting the progressive and devastating nature of ALS.

Key Words ALS · amyotrophic lateral sclerosis · neuroinflammation · inflammation · neurodegenerative disease

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Overview of Motor Neuron Injury in Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is the most prominent adult motor neuron disorder, affecting mostly motor neurons in the cerebral cortex, brainstem, and spinal cord [1]. Although first clinically described by Charcot in 1874, much of our understanding of the pathophysiology has occurred in the last 2 decades [2]. In 1993, the first genetic cause in ALS was identified, a point mutation in the gene encoding Cu^{2+}/Zn^{2+} superoxide dismutase (SOD1). The following year, the human cassette containing the G93A mutation of SOD1 (mSOD1) was inserted into a mouse [3–5]. Surprisingly, these mSOD1 transgenic mice developed a motor neuron disease similar to human ALS. Currently, > 150 genetic mutations and > 20 different genes have been identified that can lead to the same clinical disease of ALS in patients [6, 7]. Thus, multiple mechanisms converge leading to inflammation and selective motor neuron death (Fig. 1).

The study of patients in combination with the ALS animal model has identified many complex molecular and cellular

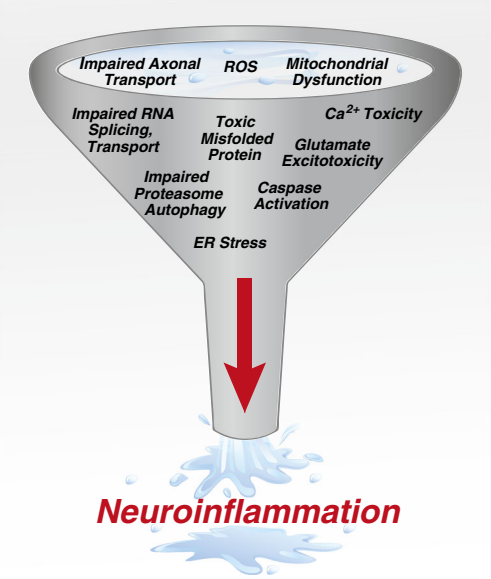

Fig. 1 Mechanisms of motor neuron injury. Motor neuron injury occurs in amyotrophic lateral sclerosis through both cell autonomous- and noncell-autonomous pathways. Multiple mechanisms can lead to motor neuron injury, which funnel into a final pathway or noncell-autonomous toxicity and neuroinflammation leading to motor neuron death

injurious pathways occurring peripherally at the neuromuscular junction and centrally at the cell body of the motor neuron [8–24]. Histopatholgy in the animal model reveals a disruption at the neuromuscular junction that appears first followed by a "dying back" of the axon [25]. Postmortem examination of ALS patient tissues reveals an obvious loss of motor neurons in the central nervous system (CNS), while many remaining neurons demonstrate central chromatolysis, numerous inclusions, swelling of the perikaryon and proximal axon, mitochondria swellings, vacuoles, and neurofilament accumulations [26]. Intracellular inclusions of ubiquitinated misfolded proteins, including transactive response DNA binding protein 43 kDa, SOD1, phosphorylated neurofiliaments, fused in sarcoma, and/or cystatin C occur in both hereditary and nonhereditary cases, and are a pathologic hallmark of disease [27–32]. The processes involved in motor neuron injury can be further categorized into mechanisms that are "cell autonomous" occurring within the motor neuron, and "noncell autonomous" involving multiple non-neuronal cells contributing to the disease process [33].

Evidence for immune and glial cells (i.e., noncell-autonomous injury) affecting the fate of motor neurons comes from the mSOD1 transgenic mouse. In chimeric mice with selective expression of mSOD1 in motor neurons, an ALS phenotype either does not develop or develops very late in the life of the mouse [34–36]. Similarly, selective mSOD1 expression in non-neuronal cells also does not lead to the ALS phenotype, but motor neurons can develop signs of injury [37–40]. The addition of mSOD1-expressing astrocytes and microglial cells has been shown to accelerate disease progression, and wild-type microglia and astrocytes have been shown to slow disease [37, 39, 41, 42]. Other non-neuronal cells such as oligodendrocytes have also been shown to contribute to motor neuron injury, although through noninflammatory mechanisms [43–45]. To summarize the last 20 years of experimental manipulation of transgenic animals, ALS appears to be a multifactorial disease with many mechanisms leading to injury, but requiring non-neuronal cells for rapid disease progression and motor neuron death.

Much of the noncell-autonomous toxicity can be defined as "neuroinflammation". Once thought to be only a consequence of motor neuron death, neuroinflammation is now established as an important factor not only in the pathogenesis of ALS, but also in many other neurodegenerative diseases, including Parkinson's, Alzheimer's, multiple sclerosis, HIV-associated encephalopathy, and cerebrovascular disease [46–51]. Much of what has been learned in ALS regarding neuroinflammation has come through discoveries in these other neurodegenerative diseases. Neuroinflammation is now understood to contribute to the balance between neuroprotection and neurotoxicity. Evidence of the dual nature of inflammation in ALS exists in both human patients and animal models. During periods of slow disease progression, an anti-inflammatory process governs neuroinflammation. During periods of rapid progression, however, neuroinflammation is governed by a strong proinflammatory state. In this review, we discuss the key cellular players, the early neuroprotective phase (Fig. 2) and late injurious responses (Fig. 3), and potential treatment strategies to modulate these responses.

Immune Cells Involved in the Neuroinflammation of ALS

Microglia

Microglia are considered to be one of the first lines of defense for the CNS against injury and infection. As a component of the innate immune system, microglia colonize the CNS during early development to serve as the resident macrophages [39, 52]. Microglia, in addition to sampling the environment and presenting antigen, have patterned recognition receptors such as CD14 and Toll-like receptors (TLRs) 2 and 4, that when stimulated result in an innate immune response. These receptors have been suggested to be involved in multiple diverse neurodegenerative diseases [51, 53, 54]. Depending on the stimulus and surrounding cytokine milieu, microglia can be activated along a continuum with the ability to acquire a classically activated (M1) or alternatively activated (M2) phenotype [54–58]. In general, M1 promote a neurotoxic T-cell response and are cytotoxic owing to the secretion of reactive oxygen species (ROS) and proinflammatory cytokines, including interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α, and a reduction in protective trophic factors

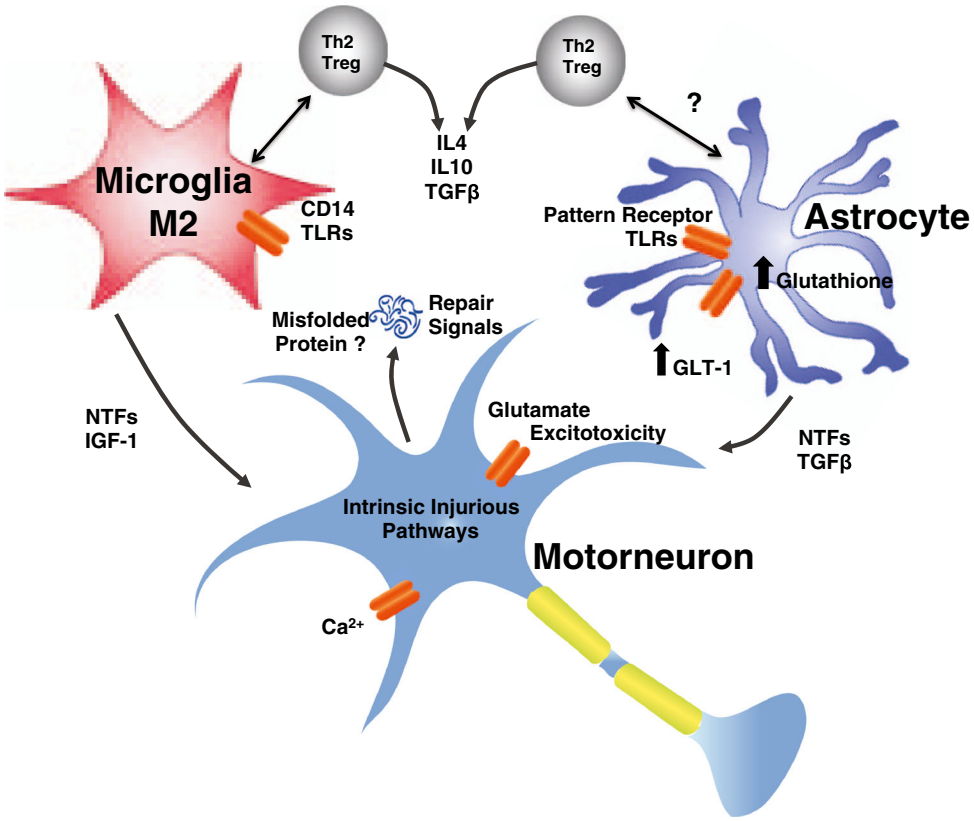

Fig. 2 Neuroprotective phase of neuroinflammation. Initially in the disease course of amyotrophic lateral sclerosis, there is an early anti-inflammatory or neuroprotective compensatory response of surrounding glia and immune cells. This early response is governed by T helper 2 cells (Th2)/regulatory T lymphocytes (Tregs), M2, and supportive astrocytes secreting neurotrophic factors and decreasing neuronal stress. GLT-1 = glutamate transporter; NTFs = neurotrophic factors

[59–63]. In contrast, M2 produce high levels of anti-inflammatory cytokines and neurotrophic factors including IL-4, IL-10, and insulin-like growth factor (IGF)-1 in addition other neruoprotection signals such as CD200 and fractalkine [51, 60, 64–68].

In patients with ALS, microgliosis occurs specifically with motor neuron injury in the motor cortex, along the corticospinal tract, and in the ventral horn of the spinal cord [24, 53, 69–72]. Interestingly, positron emission tomography imaging, with either ^{11}C-PK11195 or translocator protein

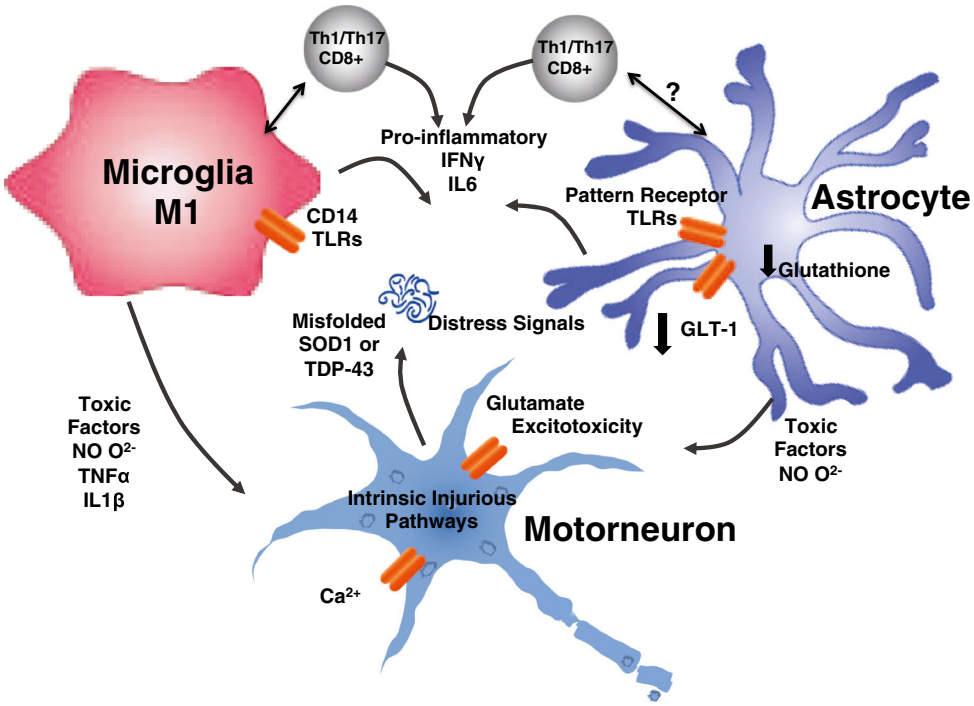

Fig. 3 Cytotoxic phase of neuroinflammation. Late in the course of the disease as the motor neuron becomes damaged, there is a transition from a neuroprotective response to an injurious response by the surrounding glia and immune cells. This is presumed to be a vicious cycle that starts when motor neuron death occurs inciting further inflammation and release of toxic factors

radioligands that bind microglia, has been used to image microglial activation corresponding with the location motor neuron injury in patients with ALS [72, 73]. This radiographic evidence in humans correlates the with evidence in the G93A mSOD1 transgenic mouse, which demonstrates microglial activation even before motor neuron cell death [74, 75].

Astroglia

Astrocytes have many complex functions, including regulating extracellular neurotransmitter concentrations, exerting metabolic or ionic homeostatic function, providing structural and trophic support for neurons, and also contributing to the immune response [76, 77]. Astroglia contribute to motor neuron injury in both immune and nonimmune mechanisms. In patients with ALS and in animal models, astroglia have diminished expression of glutamate transporter-1 (excitatory amino-acid transporter-2), leading to glutamate excitotoxicity [10, 78, 79]. Astrocytes can also contribute to the immune response via pattern recognition receptors, including TLRs and mannose receptors. Following activation, astrocytes can secrete neurotoxic factors and cytokines, such as C-X-X motif chemokine 10, chemokine (C-C motif) ligand 2, and IL-6, greatly affecting the local environment [24, 40, 53, 76, 80].

In patients with ALS, astrogliosis occurs more diffusely than microgliosis, occurring in the spinal cord as well as in the gray matter and subcortical white mater [81, 82]. In the G93A mSOD1 transgenic mouse, activation of astrocytes occurs concomitantly with a decrease in motor neurons [75, 82, 83]. Although astrocyte numbers increase with disease progression, astrocytes do not proliferate like microglia, but may be derived from ependymal cells lining the central canal of the spinal cord or oligodendrocyte precursor cells [24, 84–88].

Additional Local Immune Cells

Local dendritic cells (DCs) have been less well characterized, but also serve an important function in the disease process. DCs sample the local environment and present foreign antigens, and are increased in both human patients with ALS and in the mSOD1 transgenic mouse [89, 90]. During the course of disease, microglia/macrophages, DCs, and T cells contribute to the cytokine milieu that dictates the local neuroinflammatory response.

T Lymphocytes

T cells play an important role in governing acquired immune responses to diverse antigens. Specific T-cell subpopulations infiltrate the CNS during disease progression and contribute to the neuroinflammatory reaction in ALS [91–93]. In CNS tissues of both human patients with ALS and animal models, CD4+ T helper (Th) lymphocytes are observed in association with microglial activation, and cytotoxic CD8+ are observed in these tissues at later stages of disease [70, 93]. Several CD4+ subsets have been described, but most focus on 4 distinct subsets: Th1, Th2, Th17, and regulatory T lymphocytes (Tregs). Each subpopulation has specialized functions to control immune responses [94]. Like the classification of microglia/macrophages, CD4+ T cells can fall into 2 simplified classes: those that are neuroprotective (Th2 lymphocytes and Tregs), and those that are proinflammatory and neurotoxic (Th1 and Th17 lymphocytes). In 1995, Sakaguchi et al. [95] identified and described CD4+CD25+ immune suppressive cells with a role in self-tolerance. Tregs were then introduced to neurodegeneration with discoveries in multiple sclerosis [96, 97]. Understanding the role of Tregs play in neurodegeneration has spread, encompassing many different neurodegenerative diseases, including ALS [51, 98]. The role of Th17 is less well defined in the mSOD1 transgenic animal, but evidence from patients with ALS supports their involvement in the neuroinflammatory process [98–101].

Additional Peripheral Immune Contributions

Additional contributions from the peripheral immune system that have been investigated include the protein complement system, as well as peripheral monocytes/macrophages. Complement factors are mostly synthesized in the liver, but in mSOD1 transgenic animal models, complement factor C1q transcription was noted to be upregulated in motor neurons [102]. However, the role of the complement pathway still remains controversial [103–106]. The role of peripheral monocytes in ALS is also controversial. Several authors suggest that peripheral monocytes infiltrate the ALS spinal cord contributing to motor neuron loss [107, 108]. These results are inconsistent with previous parabiosis experiments that demonstrated no infiltration into the CNS from the periphery unless the blood–brain barrier was disrupted [84, 109–112]. However, monoctyes/macrophages have been demonstrated to aid in the inflammatory response of peripheral axons [113]. Additionally, immune system alterations, including decreased CD14+ monocytes early in the disease course, as well as evidence of monocyte activation in the blood, are documented in patients with ALS [114]. Further investigation is needed in both areas to define their contribution to neuroinflammation in ALS.

Immune Cell Dialogue

During periods of neuroinflammation, microglia acquire properties of antigen presenting cells such as CD11c, CD86, and intracellular adherin molecule 1, suggesting that these microglia interact closely with T cells [84, 89, 90, 92]. *In vitro* studies demonstrate that M2 cells have the ability to induce $CD4^{+}$

Tregs with a strong suppressive anti-inflammatory function [115, 116]. Similarly, CD4+ T cells cultured with M1 macrophages can produce a proinflammatory interferon (IFN)-γ response [117]. T cells can also polarize microglia toward a M2 neuroprotective or a M1 cytotoxic phenotype depending on the type of T cell and cytokine milieu [67]. Additionally, all cytokine-producing cell types release factors that can influence activation states of the others [118–120]; chemokine (C-C motif) ligand 2 and macrophage colony-stimulating factor are among other factors that are secreted by astrocytes and microglia in ALS [121]. It is thought that damaged motor neurons initiate the inflammatory response, although the exact mechanism is unknown. Potentially, mSOD1 released by motor neurons activate astrocytes and microglia via a TLR response [122, 123]. Intriguingly, mSOD1 has been demonstrated to transform and activate microglia to a M1 proinflammatory state, but mSOD1 also can induce a neuroprotective state with production of IGF-1 and progranulin secretion in certain conditions [53, 122, 124–127]. Both of these states can exist during the course of ALS and are determined by the cytokine milieu created in response to motor neuron injury.

Early Neuroprotective Neuroinflammation Phase

The initial belief regarding neuroinflammation in ALS was that all the cellular players represented a neurotoxic state. This idea was challenged after transfer of wild-type microglia slowed disease progression in the mSOD1 mouse [39]. Similarly, the view of T cells in ALS was changed by experiments that depleted the entire T-cell population by crossbreeding the mSOD1 transgenic mice with $RAG2^{-/-}$ knockout or $TCR^{-/-}$ knockout mice. Surprisingly, the disease course significantly worsened in these mSOD1 Tcell-deficient mice [92, 93]. Transfer of CD4+, and specifically Tregs, rescued these mice and extended survival [93, 128]. Th2 CD4+ T cells and Tregs can express high levels of the anti-inflammatory factor IL-4, polarizing microglia to the neuroprotective (M2) phenotype [92]. Furthermore, these "neuroprotective" T cells may influence astroglial behavior by increasing their production of neurotrophic factors such as glial-cell-derived neurotrophic factor [93].

In the mSOD1 transgenic mouse, after disease onset, 2 clinical phases exists: An initial "slow" phase where the mouse does not appear to worsen clinically, followed by a "rapid" phase where the mouse declines clinically until euthanasia. In 2011, Beers et al. [98] demonstrated in the G93A mSOD1 transgenic mouse that during this initial "slow" phase M2 factors were predominant (Fig. 2). Also during this early phase, Th2/Tregs predominate with the secretion of IL-4, IL-10 and other anti-inflammatory cytokines [98, 129]. Similarly, when Tregs were passively transferred into the ALS mouse, the "slow" phase and survival were prolonged [130].

Late Neurotoxic Neuroinflammation Phase

In the transgenic mSOD1 mouse, while the Tregs/M2 dialogue actively contributes to neuroprotection during the slow phase of the disease, a transformation occurs, and a rapid phase ensues with the concomitant injurious Th1/M1 response and Treg function suppression (Fig. 3) [98]. During the rapid disease course, proinflammatory and cytotoxic T cells predominate, contributing to the neurotoxic proinflammatory environment in conjunction with production of several cytokines, such as IL-1, IL-6, TNF-α and IFN-γ [93, 98]. Overall, as the disease progresses, more Th1 cells are observed producing elevated levels of IFN-γ, which promotes M1 microglial activation. M1 cells can then promote proliferation and function of Th1/Th17 cells. This vicious cycle is believed to be a significant driving force for acceleration of disease course during the rapid phase.

Neuroinflammation in Patients with ALS: Tregs

Most importantly, many of these immune changes in the animal model have been confirmed in patients with nonhereditary ALS [24, 70, 99, 100, 131, 132]. Henkel et al. [133] demonstrated that in patients with rapidly progressing clinical states, an inverse correlation was seen between Treg numbers in the blood and leukocyte levels of FoxP3, a transcription factor required for Treg suppressive function [134]. In a second cohort of patients with ALS, the decreased cell numbers and decreased FoxP3 expression were 80 % sensitive in predicting rapid progression in patients with ALS. After 3.5 years, 35 % of patients with ALS with decreased FoxP3 levels were deceased or ventilator dependent, while only 13 % of patients with ALS with increased FoxP3 levels were deceased or ventilator dependent. Factors that are elevated during the later period that can inhibit the suppressive ability of Tregs include TNF-α, IL-1β, and IL-6. TNF-α has been demonstrated to inhibit the phosphorylation of FoxP3 [135]. IL-1β was required to drive the conversion of Tregs to IL17-producing cells [136]. IL-6 has been reported to inhibit the generation of FoxP3+ Tregs [137]. Interestingly, anti-IL-6 treatment through toclilizumab has shown suggestive clinical benefit and is currently being tested in patients with ALS [138]. Clearly, understanding the interactions between peripheral and central immune responses will be essential in any attempt to manipulate the disease via neuroinflammatory mechanisms.

ALS Immunomodulation Treatment Strategies

In the last decade, neuroinflammation has been documented to contribute to the pathogenesis of motor neuron injury in

transgenic ALS mouse models. No therapy that appeared promising in transgenic ALS mice, including many targeting neuroinflammation, has improved clinical outcomes in patients with ALS. It is clear that the mouse model has a more homogenous disease phenotype compared with the heterogeneous clinical disease in patients; humans are not just big mice. Multiple factors provide insight as to why translation of therapeutic benefit from mouse to human has failed, including the lack of data delineating the extensive cytokine milieu created in response to motor neuron injury. In mSOD1 transgenic mice, decreasing or deleting single proinflammatory factors such as TNF-α, IL1-β, and inducible nitric oxide synthase has had little-to-no effect on overall survival [139–141]. Clearly, the multiplicity of proinflammatory cytokines can compensate for the absence of any single factor, and it is unlikely that continuing efforts to target a single factor will provide significant therapeutic benefit in patients with ALS.

Drugs targeting neuroinflammation such as celecoxib, ceftriaxone, thalidomide, and minocycline were reported to enhance survival in transgenic mice, yet none were effective in human ALS trials [142–144]. Some critics have suggested suboptimal animal study design such as inadequate powering or administration of drug to mice prior to onset of signs of disease, while in patients with ALS administration can only begin well after the appearance of disease symptoms and signs [145, 146]. Similarly, targeting the downstream effect of ROS has shown benefit in ALS animal models but not in patients with ALS [147]. Immunosuppressive drugs such as glucocorticoids, cyclophosphamide, azathioprine, and cyclosporine, among others, that have proven efficacy in diverse immunological disorders have not shown efficacy in ALS [148, 149]. If neuroinflammation is an appropriate therapeutic target in ALS, we must ask why these immunomodulatory medications have failed in clinical trials. Is it too little too late? Is neuroinflammation a meaningful therapeutic target only in a small population of the diverse ALS phenotypes? Or is it possible that these therapies failed to hit the appropriate targets? Unfortunately, in most studies, neither the appropriate target is defined nor is there evidence that the therapies actually hit the putative target.

The lack of translation of therapeutic benefit in transgenic mouse models to patients with ALS underscores at least 2 complicating factors: the significant heterogeneity of disease in patients with ALS, and the lack of biomarkers to differentiate patients with ALS with slowly progressing disease from those with rapidly progressing disease. In mSOD1 transgenic mice, early slowly progressing disease can be readily differentiated from later rapidly progressing disease; the spinal cords of the former are characterized by increased protective Tregs and M2 microglia/macrophages, while the spinal cords of the latter are characterized by decreased Tregs and increased Th1 lymphocytes and M1 microglia/macrophages. However, in the heterogeneous population of patients with ALS enrolled in any study, populations of patients progressing rapidly are intermixed with patients progressing slowly. Even though neuroprotecitve immune factors may be present in the spinal cord in early disease and neurotoxic immune factors in later disease in patients with ALS, readily available access to spinal cords, disease heterogeneity, and an inability to separate slow from fast progression significantly limit therapeutic benefit. In the absence of the ability to separate fast from slow progressors, or the presence of an admixture of neuroprotective and neurotoxic cellular immunity, therapeutic efforts may need to simultaneously downregulate proinflammatory cellular immunity and upregulate anti-inflammatory cellular immunity.

One potential target and treatment strategy would be to decrease the signals activating the inflammatory response. This approach includes the use of interfering RNA to decrease production of misfolded toxic proteins. These techniques have lead to success and positive results in animal models [150, 151]. Currently, this approach is being translated in the clinic to patients with ALS; however, no long-term outcome data have been published [152]. There is concern, though, that once the vicious, irreversible proinflammatory cycle begins, this approach may not be successful.

To modulate effectively neuroinflammation in ALS, the studies in transgenic mice suggest potential targets that are not modulated by present-day anti-inflammatory or immunosuppressive therapies. During early stages of disease in the mSOD1 mouse, microglia are neuroprotective (M2), while in later, rapidly progressive stages of disease microglia are cytotoxic (M1). T lymphocytes are similarly neuroprotective early in disease (Th2/Tregs) and neurotoxic later in disease (Th1/Th17). Therapies that target all populations of T lymphocytes, including Tregs, Th2, Th1, and Th17 cells, would simultaneously suppress both protective and cytotoxic populations. Thus, potentially effective therapies would need to enhance protective T cells (Tregs/Th2) and/or suppress cytotoxic T cells (Th1/Th17), but suppressing both populations simultaneously would not change the balance between pro- and anti-inflammatory responses, and might not promote beneficial effects. Similar therapeutic strategies targeting microglia or monocyte/macrophage populations should preferably attempt to enhance microglial M2 responses and suppress microglial-mediated M1reponses simultaneously.

The successful transfer of Tregs into ALS mice to upregulate neuroprotective pathways and downregulate cytoxic pathways makes therapies targeting Tregs attractive. Passive transfer of Tregs has become an effective clinical therapy in graft-versus-host disease [153, 154]. Treatment with cytokines and

growth factors such as IL-2, granulocyte macrophage colony-stimulating factor, or IGF-1 have been shown to increase Tregs reliably in other diseases [155–157]. Epigenetic modification of *FOXP3* is also under investigation for potential treatment opportunities [158]. Future therapies in ALS involving Tregs may incorporate all these approaches to tip the balance back towards neuroprotection. One significant caution in Treg therapy is the potential of these cells to be converted to Th17 proinflammatory cells in the presence of the increased proinflammatory cytokine milieu, which may promote disease progression.

Other cellular based therapies targeting immune cells are also under investigation. Early attempts with CD34 hematopoietic stem cell transplantation were not successful at enhancing survival or suppressing the neuroinflammatory responses within the spinal cord, for reasons mentioned above [159]. However, transplantation of mesenchymal stem cells has had promising results in animal models, and early-phase trials in patients with ALS are in progress [160]. In these later studies, the goal is not to replace the injured motor neuron, but to use these stem cells as a Trojan Horse to deliver growth factors to repair motor neurons and thereby halt disease progression. Another cell-based therapy to repair injured motor neurons is to inject embryonic stem cells directly into the spinal cord of patients with ALS; to date, 18 patients with ALS have been implanted and are being carefully monitored [161, 162].

Another approach to suppressing neuroinflammation would be to decrease the population of M1 monocyte/macrophages and enhance the population of M2 monocyte/macrophages. Similar to Tregs, passive transfer may be an option. Transfer or M2 microglia/macrophages has been shown to be an effective treatment in experimental autoimmune encephalitis [116]. Already in clinical trial, NP001, a novel immunomodulator of proinflmmatory monocyte/macrophages, appeared to suppress neuroinflammation and slow progression in ALS, but these results await a larger confirmatory study [163].

Conclusions

Effective therapy of ALS remains in its infancy, even after many years of intense investigation and discovery. As highlighted throughout this review, the pathways involved in motor neuron death in ALS are complex, with diverse cellular and molecular contributions leading to motor neuron injury and eventual neuronal death. Neuroinflammation in neurodegenerative disease has evolved from what was once thought as a secondary effect or consequence of neuron injury to being accepted as making a key contribution to motor neuron pathophysiology and disease propagation. In ALS, the fact that mutations in different genes can result in common clinical manifestations of ALS, as well as the fact that neurons do not die on their own, suggest that neuroinflammation is a common denominator and necessary to induce neurodegeneration in ALS.

Despite the complexity, disease progression in ALS that eventually leads to motor neuron death can be divided into 2 phases. The first phase is cell autonomous, with motor neuron injury mediated by many mechanisms summarized in Fig. 1. During this early phase of slow disease progression, data gleaned from both human and animal models suggest that the immune system is neuroprotective with glia and T cells, especially M2 macrophages/microglia and Tregs, providing factors that sustain motor neuron viability (Fig. 2). As the disease progresses and the intrinsic motor neuron autonomous injury proceeds and accumulates, and the extrinsic noncell-autonomous repair processes fail and a second rapidly progressing phase ensues characterized by M1 macrophages/microglia, and Th1 and Th17 T cells. Although the signals emitted from motor neurons triggering both an injurious innate immune glia and adaptive immune T-cell response have not been fully elucidated, current data suggest that the neuroprotective M2/Treg/Th2-mediated pathways are downregulated and the cytotoxic M1/Th1/Th17 pathways are upregulated, resulting in a self-propagating proinflammatory acceleration of disease progression. The nature of these signals remains unknown, but the misfolded proteins themselves, such as SOD1 and transactive response DNA binding protein 43 kDa, or peptide fragments may mediate an M1/Th1/Th17 proinflammatory cascade leading to the precipitous demise of the motor neuron. Such neuroinflammation may not initiate neuronal injury, but amplify and propagate the injury instigated by the motor neuron-emitted “danger signals”.

Despite many studies defining the multiple intraneuronal pathways compromised in ALS, no therapies have provided meaningful benefits to patients with ALS. Current data suggest that cell-based therapies aimed at affecting and modulating the neuroinflammatory responses in ALS might provide these therapeutic benefits. Tregs are especially attractive as a potential therapy as the passive transfer of Tregs in the mSOD1 mouse model of ALS demonstrated clinical improvement and prolonged survival. Alternative therapies include compounds that can maintain the early microglial M2 phenotype and other compounds that transform the late microglial M1 phenotype into a protective M2 phenotype. Thus, a focus on cell-based therapies aimed at modulating the neuroinflammatory response in ALS, including the specific signals involved in the microglial–T-cell dialogue, may help arrest the progressive and devastating nature of this disease, and provide hope for patients with ALS.

Required Author Forms Disclosure forms provided by the authors are available with the online version of this article.

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