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Disease origin and progression in amyotrophic lateral sclerosis: an immunology perspective

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Abstract

The immune system is inextricably linked with many neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), a devastating neuromuscular disorder affecting motor cell function with an average survival of 3 years from symptoms onset. In ALS there is a dynamic interplay between the resident innate immune cells, i.e. microglia and astrocytes, which may become progressively harmful to motor neurons. While innate and adaptive immune responses are associated with progressive neurodegeneration, in the early stages of ALS immune activation pathways are primarily considered to be beneficial promoting neuronal repair of the damaged tissues, though a harmful effect of T cells at this stage of disease has also been observed. In addition, while autoantibodies against neuronal antigens are present in ALS, it is unclear whether these arise as a primary or secondary event to neuronal damage, and whether the autoantibodies are indeed pathogenic. Understanding how the immune system contributes to the fate of motor cells in ALS may shed light on the triggers of disease as well as on the mechanisms contributing to the propagation of the pathology. Immune markers may also act as biomarkers while pathways involved in immune action may be targets of new therapeutic strategies. Here, we review the modalities by which the immune system senses the core pathological process in motor neuron disorders, focussing on tissue-specific immune responses in the neuromuscular junction and in the neuroaxis observed in affected individuals and in animal models of ALS. We elaborate on existing data on the immunological fingerprint of ALS that could be used to identify clues on the disease origin and patterns of progression.

Introduction

Motor Neuron Disease (MND) encompasses a wide range of pathological entities in which the clinical presentation depends on the extent and anatomic location of motor cell loss (1). One variant
of MND is amyotrophic lateral sclerosis (ALS), first described by Jean-Martin Charcot in the 1800s. ALS is a neuropathological entity characterised by progressive degeneration of motor neurons in the cortex, brainstem and spinal cord. The onset of ALS is generally observed in late adulthood although juvenile forms do occur albeit rarely (2,3). ‘Amyotrophic’ refers to muscular atrophy, and, ‘lateral sclerosis’ pertains to the scarring in the lateral aspect of the spinal cord. ALS is a good illustration of clinical heterogeneity; the progression of the disease, exemplified by the development to paralysis, swallowing impairment and respiratory failure may occur within months or years (4). Dominant clinical features, including muscle wasting and weakness, may be accompanied by or preceded by clinical manifestations of frontotemporal dementia (FTD) (5-7).

Genetic mutations have been described mostly in autosomal dominant familial cases of ALS, including the cytosolic anti-oxidant enzyme Cu²⁺/Zn²⁺-binding superoxide dismutase 1 (encoded by SOD1) (8) trans-activation response element (TAR) DNA-binding protein 43 (TDP-43; encoded by TARDBP) (9), fused in sarcoma RNA-binding protein (encoded by FUS) (10), angiogenin (encoded by ANG) (11) and more recently, an intronic hexanucleotide expansion in the gene encoding the chromosome 9 open reading frame 72 (C9orf72) (12). Increasing knowledge of the role of these genes has changed the landscape of ALS research and allowed further insight into the core pathological mechanisms operant in the development of the disease.

The clinical course of most ALS cases follows a steep curve of deterioration with a rapid and sometimes unpredictable progression to respiratory failure and to the demise of bulbar functions (loss of function of muscles involved in speech and swallowing) (4). Patients also present with muscle fibre spontaneous activity (fasciculations), weakness and muscle wasting, signs of cortical (upper) motor neuron involvement and cognitive impairment where behavioural changes of the FTD type predominate (11). Lower motor neuron involvement is potentially the biggest contributor to the neurological decline, affecting the rate of progression to end-stage life-threatening events,
clinical milestones such as respiratory failure, the loss of the ability to swallow and the risk of aspiration.

The average delay of 15 to 18 months from onset of symptoms to diagnosis that relies on clinical and neurophysiological observations, depends also on the subtleness of the initial clinical manifestations, which may not be disabling enough to be fully appreciated by the patients or by the examining physicians (13,14). The lack of disease-specific biomarkers, to detect early pathology when ALS is suspected, or as disease monitoring tools, further complicates matters. This ‘diagnostic latency’ reduces the therapeutic window for potential neuroprotective therapies, while neuron-rescuing strategies may lose their greatest chance to work. Further uncertainties surround the definition of the site of initiation of the disease in ALS; whether this is at the neuromuscular junction with an upward development (lower motor neuron first) or the corpus callosum and cortex with a rostro-caudal (front-to-back) progression (upper motor neuron first) (15-17).

Various promising biomarkers for ALS are currently being investigated but none of these are disease-specific or informative enough to allow further speculation on the disease origin or to be used as prognostic indicators (18). One potential strategy to depict early disease and to monitor its progression makes use of the immune response that is activated during the pathological process. Here, we discuss the role of the immune pathological changes associated with ALS onset and progression. We elaborate on the potential triggers of immune responses in ALS and in experimental animal models that recapitulate in part the human pathology. Finally, we discuss how the immune changes may act as biomarkers of disease, and how targeting the immune response could provide novel disease-modifying therapies.

Immunology of ALS
The immune response is broadly defined as the ability of an organism to resist disease through the activities of innate and adaptive immune systems. External as well as intrinsic stressors, endogenous pathological events or changes in homeostasis may trigger such immune responses. Accumulating evidence indicates that immune activation is observed in many neurodegenerative disorders (19,20), including ALS, and may arise as a consequence of protein misfolding and aggregation, oxidative stress and glutamate excitotoxicity among others (21,22). Evidence for immune activation is observed in biological fluids including blood and cerebrospinal fluid (CSF); these immunological signals may reflect disease progression and could thus act as biomarkers of the disease.

Evidence for the role of immune responses in ALS is primarily based on post-mortem studies that offer only a late and limited snap-shot of this evolving pathological process. In the central nervous system (CNS), the presence of activated microglia at the site of motor neuron damage has been reported in neuropathological studies and using imaging techniques (23). CD8\(^+\) cytotoxic cells and CD4\(^+\) T-helper cells are also observed in the ventral horns of the spinal cord, in the anterior and lateral corticospinal tracts and in the motor cortex (24-27). The cascade of molecular events that accompanies the innate immune response may be one of the main driving forces in the unravelling of the pathological process in ALS.

**The priming event in ALS pathology: the role of the immune system**

Genetic discoveries and the use of animal models have only started to uncover some of the facts surrounding the aetiology of ALS. The study of the immune response linked to the disease may enable a better understanding of the sequence of events leading to motor cell loss, while also representing an exploitable disease biomarker.

Glial cells (e.g. microglia and astrocytes) are non-neuronal cells that support and protect neuron homeostasis. It is proposed that in ALS immune cells, such as microglia—the brain-resident macrophage population deriving from monocyte precursors—become activated in the affected tissues in response to a priming event e.g. misfolded and aggregated proteins (9,28,29). In ALS, like
other neurodegenerative and CNS proteinopathies, as well as in senescence, misfolded proteins may trigger inflammation via nuclear or cytosolic damage-associated molecular pattern proteins, also known as danger-associated molecular pattern molecules (DAMPs). These altered states may trigger a state of chronic activation of the innate immune system. Pattern-recognition receptors (PRRs) expressed by different types of immune cells, sense these changes and may mediate the immune cell activation (30,31). As reported for other neurodegenerative diseases, DAMP signalling likely participates in the neurodegenerative process in ALS. DAMP molecules including reactive oxygen species (ROS), heat-shock proteins (HSPs), and high-motility group box 1 (HMGB1) are reported to be overexpressed in the spinal cord and in the motor cortex from mice that over-express mutant forms of the human SOD1 gene (hmSOD1) and from patients with ALS (32-35). A recent study shows that the eosinophil-derived neurotoxin (EDN), a DAMP-like molecule, is also elevated in sera from patients with ALS and may have relevance in the pathophysiology of this disorder (36).

The question of whether the origin and propagation of the immune response is predominantly peripheral (i.e. at the neuromuscular junction (NMJ) and axons) or central (motor cell-containing areas) is critical for the overall understanding of the disease, for the development of novel treatments and for biomarker discovery.

As the disease progresses in ALS, other leucocytes with phagocytic properties as well as antigen-presenting cells undergo the same process of activation seen in microglia (24,37-43). Dendritic cells (DCs), potent antigen-presenting cells that initiate and amplify immune responses, have also been described to have become activated in both post-mortem tissue of ALS patients and in animal models of the disease. Pro-inflammatory cytokines including interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α), interferon-γ (IFN-γ) and IL-17 are released by microglia and also by T-helper 1 (T_h1) and T helper 17 (T_h17) lymphocytes, whilst anti-inflammatory cytokines (IL-4, IL-5, IL-10 and IL-13) are released by T helper 2 (T_h2) cells (Table 1). These and other immune mediators including IL-1 receptor accessory protein (IL-1RAP) and the immune-cell-attracting chemokine RANTES are found in the proximity of motor neurons (44,45). It is questionable whether individual cytokines can have a
harmful effect in isolation, or whether a synergism is seen in co-expression, like in the case of TNF-α, IL-6 and IFN-γ (46). IL-17A seems to be specifically overexpressed in CD8⁺ cells infiltrating the ALS spinal cord and in peripheral blood from ALS patients (Table 1).

In addition to central changes, muscle and nerves appear to be the site of further immunological activation in ALS. It is not known whether brain, spinal cord and muscle are involved simultaneously or whether the pathology and related immune response unravel as a sequential process. Most immunological mediators so far implicated in the disease process are ubiquitous; hence they may be the biological effectors of a cross-talk between separate organs becoming involved in the propagation of the pathological process. The transfer of immunological signals between CNS and peripheral neuro-muscular structures or the flow of the same immune mediators in the opposite direction may also be facilitated by the breakdown of the blood–brain barrier (BBB). Both BBB and blood–spinal-cord barrier (BSCB) are reported to be compromised in an animal model of ALS due to endothelial cell degeneration (47,48).

The immunological microenvironment of the neuromuscular compartment in ALS patients, with muscle showing hyper-excitability and undergoing atrophy, has not been fully characterised in the progression of the disease. Recent experimental evidence demonstrates the accumulation of macrophages expressing CD11b and CD68 in peripheral nerves in the hmSOD1 transgenic mouse model of ALS (49,50) (Figure 1). Studies in transgenic mice suggest that the muscle actively contributes to the disease onset and has an active role in motor neuron degeneration, probably by producing factors that inhibit neurite outgrowth. Investigations into ALS animal models have shown that motor neuron death can be preceded by NMJ destruction and distal axonal degeneration (51).

Muscle denervation, a critical feature in the clinical progression of ALS, depends on a variety of factors, each with a particular mode of action. The activity of acetylcholine receptors (AChRs) for
example seems to play a role in the innervation and re-innervation of muscle fibres (52) while the expression of insulin-like growth factor 1 (Igf-1) has been implicated in anabolism of muscle and nerve tissues survival (53). Induction of Igf-1 in muscle has been shown to stabilize NMJs, reduce inflammation in the spinal cord and enhance motor neuronal survival in hmSOD1 mice, delaying the onset and progression of the disease (54). Data on primary muscle involvement in ALS pathology are controversial. Reduction of mutant SOD1 synthesis in the skeletal muscle of ALS mice does not affect disease onset or progression (55) although the expression of the mutated SOD1 gene under the transcriptional control of muscle-specific promoter in transgenic mice demonstrate that skeletal muscle is a primary target of SOD1-G93A mediated toxicity (56).

**Disease initiation and propagation in ALS: an immunological perspective**

In neurodegenerative disorders, the onset and propagation of the disease is likely to pre-date the stage when the disease becomes symptomatic. In ALS, experimental evidence indicates that the muscle and the NMJ may be the site of disease manifestation in a very early stage. In animal models of the disease, gene-expression changes in muscle predate any overt motor neuron involvement (56). In the human pathology, neurophysiological studies have portrayed signs of denervation, NMJ alterations and spontaneous activity from a very early and mildly symptomatic stage of the disease (15). In contrast to this, more recent suggestions from studies performed using structural Magnetic Resonance Imaging and tractography suggest a predominant rostro-caudal or cortico-spinal progression of the disease in ALS individuals (57). Given the lack of a recognisable pre-clinical state in ALS, it is ultimately difficult to define an exact temporal sequence of events and the direction of the pathological spread in ALS.

The expression of factors involved in the immune response in muscle and more specifically at the level of the NMJ is also generally described as an early feature of the disease. In muscle biopsies from ALS patients, antibodies directed against NMJ protein epitopes expressing P/Q-type calcium
channels (Figure 1) have been demonstrated along with neural cell adhesion molecule (N-CAM)⁺ myofibers (58).

Anti-GM1-ganglioside antibodies, accumulating near the nodes of Ranvier, have been shown to be relatively specific to patients with a disease of the lower motor neurons (59) and, although rare, antibodies against AchRs are found to increase in serum from ALS patients, most likely in response to the degeneration of AchR at the NMJ (60). A recent study shows that antibodies to LRP4, a postsynaptic membrane protein of the NMJ and motor neurons in the brain and spinal cord, may also have a direct pathogenic activity in ALS by participating in the denervation process (61) (Table 2, Figure 1).

Cytokines like IL-6, IL-8, IL-15, TNF-α, macrophage inflammatory protein 1 (MIP-1), IL-1, IL-1 receptor antagonist (IL-1ra), IL-10, IL-4 and IL-13 can be produced in a physiological state by contracting muscle fibres and have an up-ward trend of expression following strength training. Cytokines are central to the muscle metabolic machinery. IL-6, for example, induces glucose uptake and fatty acid oxidation in muscle, stimulates hepatic gluconeogenesis and induces lipolysis (62,63). Similarly, IL-15 is involved in muscle–adipose-tissue crosstalk (64), whilst high local IL-8 concentrations might be involved in exercise-induced angiogenesis and increased capillarization of skeletal muscle (65).

Equally, ALS has been linked to the up-regulation of most of these cytokines in affected tissues and peripheral blood, including IL-6, TNF-α and TGF-β1 among others. For example, longer disease duration and end-stage disease have been positively correlated with higher TGF-β1 levels (66,67). In the human pathology, a conclusive relationship between rate of disease progression or stage and TNF-α levels in biological fluids has not been established (26, 68-70). IFN-γ and its downstream factors CXCL10 and MIP-1β may be an example of a protective response in neurodegeneration that can also be measured in the CSF. These markers were negatively correlated with the rate of disease progression in one study, in line with their supposedly neuroprotective function (70), but not conclusively linked to any particular disease state or rate of progression in some others (46,71-74).
The change in the inflammatory environment in ALS has also a chemo-attractant effect whilst enhancing cell proliferation. The chemokine MCP-1, which attracts peripheral immune cells into the CSF, is overexpressed in CSF from ALS patients compared with controls. A positive correlation between increased MCP-1 and IL-8 in biological fluids and a more rapid disease progression [or a negative correlation with a poorer functional status e.g. the decline of the ‘revised amyotrophic lateral sclerosis functional rating scale’ (ALSFRS-R)] has been suggested but not conclusively established (70,75-77). The expression of monocyte proliferation factor increases with the build up of microgliosis in the late stage of the disease (71,74).

Understanding the specific tissue origin of these cytokines in ALS patients who may undertake a variable degree of physical activity may help understanding the immunological fingerprint of the disease and provide more information on the temporal sequence of pathological events in the development of the disease. Muscle exercise not only affects muscle plasticity but also may set in place a positive inflammatory response that may mitigate the toxic inflammatory cascade linked to ALS pathology. Peripheral immune responses originating from the NMJs or muscle may also spread centrally and may have a direct or indirect effect on motor cells integrity. The potential disease-modifying effect linked to the pro-inflammatory effect of muscle exercise is therefore an important area of research to study the mechanisms of the disease as well as for the development of immunomodulatory therapies.

**Does the immune response condition the rate of disease progression and survival in ALS?**

The immune response to the multifaceted and evolving pathological process in ALS may control the speed of progression of the disease by determining the rate and the extent of motor cell destruction. Studies on relatively small numbers of ALS individuals at different stages of the disease have consistently shown that the peripheral T cell population changes during the disease, in line with what previously reported in human and mouse models spinal cord specimens (78,79). In addition to an altered CD4+ /CD8+ balance, ALS displays a decrease of T-regulatory cells (T_{reg} cells) in blood
compared with healthy controls (80). Those ALS individuals where the pathology seems to progress faster have lower numbers of T\textsubscript{reg} cells in blood, suggesting a recruitment of these immunomodulatory cells from the periphery into the CNS (78-81). Declining T\textsubscript{reg} cell levels in blood in ALS may alter immune tolerance with the appearance of a more widespread autoimmunity to multiple proteins, as recently demonstrated with the increase of blood antibodies against neurofilament light chain (NfL) in advanced ALS (82).

The early activation of microglia has been flagged out as possibly exerting a protective effect on degenerating cells. This would be in keeping with the finding that T-cell deficiency and decreased microgliosis is associated with a poorer outcome in ALS animal models (83,84). However, persistent activation of microglia during the disease course is most likely to be functionally detrimental. The expression of blood CD14\textsuperscript{+} macrophages has also been found to correlate with the disease progression (85,86). There are also arguments for both a neuroprotective and for a toxic function that astrocytes may have on motor neurons in ALS and for their role as modifiers of the disease progression. Astrocytes increase pro-inflammatory activity by secreting acute-phase reactants, complement proteins and proteinases, in a concerted response classically known as “reactive gliosis”. Others suggest that astrocytes in sporadic ALS are neuroprotective as they promote glutamate uptake and reduce excitotoxicity (87).

Whatever combined effect monocytes, T cells and astroglia may have in the affected tissue, the toxic environment surrounding motor cell found in later stages of the disease relates to the predominantly pro-inflammatory milieu in affected tissues (88,89). It has been shown that the relative preponderance of T\textsubscript{h}1 over T\textsubscript{h}2 cytokines, for example, alters the balance between an overall cytoprotective environment towards a cytotoxic one and deepens the harmful cascade of neurotoxic factors like oxygen radicals, excitatory amino acids, arachidonic-acid derivatives and proteases (41,89). The upregulation of these pro-inflammatory mediators seems to occur slightly before the
transcriptional activation of apoptosis and oxidative stress-related genes in an animal model of ALS (73).

Humoral immunity in ALS

The humoral response may also have powerful disease-modifying effects in ALS. It has been shown that antibodies against 52,000- to 70,000-dalton proteins detected in brain, cerebellum, and liver as well as in spinal cord are more common in ALS than in control sera but there are no firm data on the nature of the antigens primarily targeted by this enhanced immune reactivity (90). A recent publication showed the occurrence of a panel of IgG antibodies in serum samples from ALS patients; and increased disease severity was associated with higher antibody levels (91). Likewise, IgM antibodies against modified (oxidized) wild-type (WT) SOD1 protein, described as aberrantly oxidized, have been reported in ALS patients who exhibit a longer survival compared with subjects lacking these antibodies; anti-SOD1 antibody levels did not correlate with disease severity in either the Alzheimer’s or Parkinson’s disease cohorts, suggesting that this immune response may be partially ALS-specific and have some prognostic value. In contrast, an immune response against the normal (WT) SOD1 appears to be disadvantageous in sporadic ALS (SALS), possibly because the anti-oxidizing activity of normal WT-SOD1 is overall beneficial (92).

From a different angle of observation, serum antibodies against neuron-specific antigens have been identified in patients with autoimmune diseases and in healthy individuals (59,93-99), suggesting that autoimmunity in physiological and pathological states may be enriched with activity potentially critical in neurodegeneration and in ALS. Autoantibodies may have a different impact also in relationship to the levels or avidities (82,100-102) and, within the nervous tissues, they may enter neurons and interact with intracellular targets (99) (Table 2). An increase level of anti-neuronal antibodies, targeting different components of neuronal cells, has been reported in ALS and their expression is reported to be higher in ALS patients with a more severe form of the disease compared with those with a milder condition (103).
Lessons from the immunology of ALS in animal models

In animal models of ALS, an increase of the macrophage/microglia-related cytokine TNF-α and of its receptor levels has been consistently reported during the pre-symptomatic stage (26). More importantly, measurements of the pro-inflammatory TNF-α in affected tissue and in biological fluids indicate that the level if this cytokine increases towards end-stage disease reflecting disease severity (46,71-74).

SOD1 animal models have provided further insight into the immunopathological regulation of disease progression in ALS. Unlike the significant clinical heterogeneity observed in ALS patients, ALS murine models display a predictable pattern of weakness (104). SOD1-G93A transgenic mice, overexpressing human SOD1 carrying the Gly93-Ala mutation, is the most widely used ALS model. Marked differences in the motor neuron transcriptome between two mice strains with mutant SOD1-G93A including a rapidly progressive (129Sv-SOD1G93A) and a slowly progressing form (C57-SOD1G93A) have been described. The strain-specific motor cell transcriptional profile of the C57-SOD1G93A mice with a more benign disease course consisted of a strong gene enrichment within the immune system processes compared with the more rapidly progressing 129Sv-SOD1G93A mice (105).

Beers and colleagues (26) suggest that the difference between the cervical and lumbar regions in SOD1 animal models in local expression of protective factors explains the pattern of disease progression in these animals that generally present with hind-limb involvement as an early feature of the disease. The expression of neurotrophic factors may mitigate the pro-inflammatory response in the cervical region but not in the lumbar region (26). Moreover, phagocytic microglia are significantly more expressed in the cervical than in the lumbar region at disease onset, whilst dendritic-type cells seem to be highly represented in the lumbar region. T-cell infiltration is first detectable in the lumbar region with a subsequent increase of the T,1 cytokines, IFN-γ and IL-6, as the disease progresses (106).
Other pro-inflammatory proteins such as TNF-α and IL-1β are also increased at an earlier phase in the lumbar segment and later on in the remaining spinal cord. In contrast, a Th2 response seems to dominate in the cervical region with a concordant increase of IL-4 expression. Neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factors (GDNF) are consistently more expressed in the cervical region. Treg cells are elevated in both lumbar and cervical regions from the outset and later drop to levels seen in WT littermates. These data taken together seem to suggest that the cervical region has a more beneficial immune response and a complementary expression of pro-survival factors from an early stage of the disease. These observations also support the notion of a gradual switch in the inflammatory response in the affected tissue from protective to harmful.

Immune responses as biomarkers and targets for therapies

A better understanding of the role and of the sequence of activation of immune mediators linked to ALS in affected tissues may help identify potential disease biomarkers whose expression may be tested in accessible biological fluids to provide bed-side tools for disease-monitoring. For example, both neurofilaments (NF) and antibodies against NF are present in the pathological aggregates in affected tissues where they may be detrimental to cell function and axonal transport. NF protein and autoantibodies to NF are equally over-expressed in blood and CSF from ALS patients and their relative abundance in soluble or in aggregate states may provide a marker for disease stratification based on speed of progression and duration from onset of symptoms (82,107,108).

DC activation, which has been reported to occur in the spinal cord perivascular areas from ALS individuals with a more severe disease progression, may be another source of potential signals of disease (39). The detection of DC-specific transcripts in blood as a by-product of central activation may be predominantly from fast-progressing ALS individuals and could provide a useful mean for the stratification of patients according to predicted rates of development and stage of disease. The
limiting factor may be the fact that the over-expression of DC transcripts in affected spinal cord may not be mirrored by an increase of their presence in biological fluids.

The use of ALS animal models is by far the most powerful tool to understand the origin and spread of the pathology and its immunology in ALS and to inform us about targets for novel biomarkers and/or therapeutic strategies. For example, the functional ablation of mutated SOD1 genes within microglia and astrocytes in a murine model of ALS has resulted in improved survival, indicating that these components of the innate immune response that are activated by a SOD1 gene defect may play a part in the modulation of the disease development (109). Transplantation of astrocytes with an improved glutamate transport capacity or the up-regulation of glutamate transporters in astrocytes by specific antibiotics resulted in delay of motor neuron loss and in a decline of motor functions in hmSOD1 animals (110,111).

Transgenic-SOD1 mice crossed with mice with either a deficiency in functional T cells or TCRβ showed a more rapid disease progression and shorter survival (84,112), along with the upregulation of proinflammatory cytokines TNF-α and IL-6 and the accumulation of ROS in affected tissues. Most importantly, all these changes could be reversed by a bone marrow transplantation resulting in a restored survival comparable with transgenic ALS mice without T-cell deficiency (84). However, lymphocytes at the early phase of neurodegeneration in ALS have also displayed a deleterious effect via inhibition of M2 microglial activation (113). Taken together, these findings indicate that T-cells could have a dual role, being pathogenic or neuroprotective by maintaining a balance between protective and harmful responses (26,79).

To date, immune-modulating and immuno-suppressant treatments have only been partially successful in animal models of the disease, and have been overall disappointing in patients. Response to treatments that act on the immune responses may be dependent on the stage of the disease and on the rate of progression of neurodegeneration, both conditioning the type of immunological alteration to be targeted. A personalised and stratified-medicine approach in the choice of treatment acting on the immune response is the strategy most likely to succeed. For
example, treatment with glatiramer acetate, a synthetic copolymer consisting of amino acids from
the myelin basic protein which induces a T\(_h\)2-biased and T\(_{reg}\)-inducing response, has been reported
to be effective on neurodegenerative conditions like multiple sclerosis, but has not shown any
beneficial effect in ALS patients (114). Glatiramer may in fact be more effective in ALS individuals
who are preliminarily selected based on their T-cell or T\(_{reg}\) systemic load, because of recent data
demonstrating how blood T\(_{reg}\) cell levels are reduced in ALS individuals in the early stages and with a
more severe form of the disease (80). Hence, a preliminary selection or enrichment of
immunologically homogeneous patients to maximise the chances of therapeutic success would be
necessary.

The potential of cellular therapy to modulate the activity of non-neuronal cells as well as the
transplantation of neuronal stem cells (115) and mesenchymal stem cells (116) has been used to
induce expansion of T\(_{reg}\) cells, opening new therapeutic strategies of ALS immunotherapy.
Treatments with immunosuppressive drugs including cyclophosphamide (117) and azathioprine
(118), or administration of non-specific immunoglobulins (119) have also not provided encouraging
results. This may again be due to the lack of an exact immunophenotyping of the disease, whereby
cohorts to be treated are enriched based on defined immunological fingerprints. In addition, a phase
III trial with minocycline, an antibiotic that has anti-apoptotic and anti-inflammatory effects, has
worsened the clinical picture by exacerbating the disease progression (120). However, sporadic ALS
patients that received tocilizumab infusion therapy, a drug that inhibits IL-6 signalling, showed
improvement with a reduction of clinical progression and decreased levels of inflammatory genes
(121).

Factors responsible for treatment failure or success include not only the overall effect that single
molecules may have on the cascade of immunological events central to ALS pathology, but also the
effective composition of the subgroups of patients and on the disease-related immunological state
at the time that the treatment was administered.
Conclusions

Harnessing the immune system and particularly those factors which are likely to impact on motor cell survival and on the speed of progression of this relentless process is likely to lead to novel disease-modifying strategies in ALS. From a biomarkers perspective, the choice of the most informative immune signal in blood that could inform us about the disease stage and on potentially drug-treatable targets depends on a detailed knowledge of the immunopathological changes in affected tissue. Notably, as outlined in this review, the activation of specific immune cells and mediators may provide clues to understand disease onset and progress in ALS patients, and could therefore be used for the “staging” of the disease (82).

The mobilization of the innate immune response in ALS, a rapid reaction to the unfolding pathological events, may go as far as providing valuable information on the disease state, hence help also diagnostically and inform on the likely rate of disease progression. However, this immunological signature may not be fully informative of the “when and where” of the disease. The pathological process in ALS is likely to start far in advance of the appearance of any clinical sign of disease and the profile of activated immune cells in affected tissue and in peripheral blood may only reflect a specific temporal segment of the disease. In contrast, the humoral response and the array of autoantibodies that can be interrogated may reveal pathological changes that stretch far back and give a better idea of the anatomic structures that may have been targeted and hence of the likely origin of the disease. Whilst speculative, a better understanding of the role of autoantibodies and their ability to escape immune tolerance may provide the ground for the development of disease-specific immunological signatures to be used for disease monitoring and to rate treatment response. Overall, the body of experimental evidence reviewed in this paper may help improve our understanding of the immunological framework of ALS and of the potential routes to exploit the immune response to generate novel biomarkers and to develop novel treatment strategies.

Conflict of interest statement: The authors declared no conflict of interests.
Legend

**Figure 1. Immune Involvement in ALS Inflammatory** factors like PGE, and LTB4, iNOS and NOX are expressed in mutant SOD-1 mice and patients with ALS and may participate in motor neuron death (122-124). In Brain and Spinal cord: **a)** Reactive microglia are associated with high levels of LCA, FcyRI and LFA-1, while complement receptors CR3 and CR4 and MHC-II molecules are observed in the primary motor cortex and motor nuclei of the brain stem and spinal cord (24). **b1)** Activated microglia and **b2)** infiltrating T cells (CD4⁺, CD8⁺) and NK cells occur in close proximity to degenerating corticospinal tracts in ALS and in spinal cord of SOD-1-mutant mice (125,126).

Infiltrating IL-17A⁺ CD8⁺ cells and IL-17A⁺ mast cells in the spinal cord in ALS (72). **c)** MCP-1, IL-1β and TNF-α expression precedes oxidation and apoptosis gene expression (73). In the muscle and NMJs: Changes in NMJ structure and function are observed in ALS: **d)** Over-expression of Bax and Bcl-2 at the postsynaptic domain of NMJ are associated with muscle degeneration (in ALS (127). **e)** Upregulation of inflammatory cytokines at the NMJs. **f)** Recruitment of macrophages and CD11b in peripheral nerves in SOD-1 model and ALS (49,128). **g)** Antibodies to LRP4, GM1 Ganglioside, Voltage-gated calcium channels, AchR in presynaptic motor nerve terminals and at NMJs linked to muscle denervation (50,60,61,129). **h)** Serum and CSF autoantibodies to HMGB1, GM1, Fas-R, foetal muscular proteins, vascular antigens and NF in ALS. Arrows indicate upregulation or down regulation of the different factors.

PGE, prostaglandin E2; LTB4, leukotriene B4; iNOS, inducible nitric oxide synthase; NOX, NADPH oxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; MCP-1, macrophage chemoattractant protein-1; Cox-2, Cyclooxygenase-2; LCA, lymphocyte function associated molecule-1; LCA, leukocyte common antigen; FcγRI, immunoglobulin receptor Fc gamma R1; MHC-II, class II major histocompatibility complex molecules; CD40L, CD40 ligand; NMJ, neuromuscular junction; LRP4, lipoprotein receptor-related protein 4; AchR, acetylcholine receptor; HMGB1, high-motility group box 1; Fas-R, Fas receptor; GM1, Gangliosides.
References


### Table 1. Immune factors associated with ALS and animal models of ALS

<table>
<thead>
<tr>
<th>Factors a</th>
<th>Cell / tissue expression</th>
<th>Prognostic value / rate of progression b</th>
<th>Human / animal model</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pro-inflammatory immune pathway</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO↑, Peroxynitrite↑, ROS↑ NOX2*↑</td>
<td>Neurons, astroglia, Endothelial cells, macrophages, microglia, spinal cord</td>
<td>Fast</td>
<td>Human hmSOD1 mice</td>
<td>(124,130-133)</td>
</tr>
<tr>
<td>P2X7 receptor↑ CB2, Cox-2↑</td>
<td>Astrocytes, macrophages Microglia, CNS</td>
<td>Fast</td>
<td>Human hmSOD1 mice</td>
<td>(134-136)</td>
</tr>
<tr>
<td>FasL / FasR↑, TNFL / TNFR↑ BCl2 proteins↑</td>
<td>T cells (CD8↓) Microglia</td>
<td>?</td>
<td>Human hmSOD1 mice</td>
<td>(68,127,137)</td>
</tr>
<tr>
<td>TLR2, TLR4↑ TRAIL↓</td>
<td>Microglia</td>
<td>?</td>
<td>Human</td>
<td>(138-140)</td>
</tr>
<tr>
<td>C1q↑, C4↑, C3↑</td>
<td>Microglia, macrophages</td>
<td>Fast</td>
<td>Human</td>
<td>(27,141-143)</td>
</tr>
<tr>
<td>CRP↑</td>
<td>Microglia, astroglia, endothelial cells, CSF, spinal cord</td>
<td>Prognostic indicator</td>
<td>Slow</td>
<td>Human</td>
</tr>
<tr>
<td>S100 Beta*↑ CD14*↑</td>
<td>Microglia, astroglia, CSF</td>
<td>Prognostic indicator</td>
<td>Slow</td>
<td>Human</td>
</tr>
<tr>
<td>RAGE↑</td>
<td>Microglia</td>
<td>?</td>
<td>Human</td>
<td>(138,147)</td>
</tr>
<tr>
<td>IgG↑, FcyR1↑ ICAM-1↑</td>
<td>Microglia, T cells, CNS</td>
<td>?</td>
<td>hmSOD1 mice</td>
<td>(148)</td>
</tr>
<tr>
<td>CD11b↑, CD14↑, CD18↑, SR-A↑, CD68↑, MCP-1*↑ RANTES↑</td>
<td>Microglia, monocytes, macrophages, microglia, spinal cord, CNS</td>
<td>Fast</td>
<td>Human hmSOD1 mice</td>
<td>(45,75,89,112)</td>
</tr>
<tr>
<td>DEC205↑, CD86↑, CD83↑, CD54↑, CD40↑, CD1a↑</td>
<td>Microglia</td>
<td>?</td>
<td>Human</td>
<td>(138,147)</td>
</tr>
<tr>
<td>IFN-α*↑, perforin↑</td>
<td>CD4+ T cells, CNS</td>
<td>Fast</td>
<td>Human</td>
<td>(144,145)</td>
</tr>
<tr>
<td>TNF-α*↑, IL-6*↑, IL-19↑, IL-12↑, CXCL [2,3,5] CXCL1↑, CCR2↑</td>
<td>M1 microglia, Tn1 cells, monocytes, CNS, PBMC</td>
<td>Fast</td>
<td>Human</td>
<td>(72,150-153)</td>
</tr>
<tr>
<td>IL17-A, IL-17, IL-18, IL-13↑, IL-23↑</td>
<td>CD8β, mast cells, astrocytes, macrophages, monocytes, Tn1 cells, Tn17, PBMC, CNS</td>
<td>?</td>
<td>Human</td>
<td>(72,154-156)</td>
</tr>
<tr>
<td><strong>Anti-inflammatory immune pathway</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-γ*↑, IL-10↑, TGF-β↑ CX3CR1↑</td>
<td>Microglia</td>
<td>Fast</td>
<td>Human</td>
<td>(27,149)</td>
</tr>
<tr>
<td>CD4+ FoxP3+ / Treg cells, IL-10, TGF-β↓</td>
<td>Microglia, Tn1, CNS, PNS</td>
<td>Slow</td>
<td>Human</td>
<td>(84,157,158) (67,159)</td>
</tr>
<tr>
<td><strong>Regulatory immune pathway</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4+CD25+ / FoxP3↑, IL-10, TGF-β↓</td>
<td>Microglia, Tn1, CNS, PNS</td>
<td>Slow</td>
<td>Human</td>
<td>(54,160)</td>
</tr>
</tbody>
</table>

In this column, * indicates factors associated with prognostic value or the rate of progression (fast or slow).

In this column, the associated effect is shown; ‘?’ indicates no clear association with these criteria.

Abbreviations: NO, nitric oxide; CRP, C-reactive protein; NOX, NADPH oxidase; Cox-2, cyclooxygenase-2; TRAIL, TNF-related apoptosis-inducing ligand; hmSOD1, transgenic mice expressing human mutant SOD1; Nf-L, Neurofilament light; ↑, upregulation of factors; ↓, downregulation of factors.
Table 2. Humoral and cellular response in ALS

<table>
<thead>
<tr>
<th>Type of response</th>
<th>Biofluid / tissue expression</th>
<th>Disease type / effect on disease</th>
<th>Human / animal model</th>
<th>References</th>
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<tbody>
<tr>
<td>Auto-antibody response to the target antigen</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LRP4</td>
<td>Serum, CSF, NMJ</td>
<td>Progression</td>
<td>Human</td>
<td>(61)</td>
</tr>
<tr>
<td>Voltage-gated calcium channels: P/Q-type, N type, L-type</td>
<td>Serum</td>
<td>Progression</td>
<td>Human</td>
<td>(50,129,163)</td>
</tr>
<tr>
<td>Fas-receptor (CD95)</td>
<td>Serum</td>
<td>Sporadic</td>
<td>Human</td>
<td>(164)</td>
</tr>
<tr>
<td>Vascular antigens</td>
<td>Serum</td>
<td>Sporadic</td>
<td>Human</td>
<td>(165)</td>
</tr>
<tr>
<td>Foetal muscular proteins</td>
<td>Serum</td>
<td>Sporadic</td>
<td>Human</td>
<td>(166)</td>
</tr>
<tr>
<td>GM1 ganglioside</td>
<td>Serum, CSF</td>
<td>Unknown</td>
<td>Human</td>
<td>(59,167)</td>
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<tr>
<td>HMGB1</td>
<td>Spinal cord, serum</td>
<td>Fast progression, correlation with severity of ALS</td>
<td>Human</td>
<td>(168)</td>
</tr>
<tr>
<td>Neurofilament proteins, NFL</td>
<td>Serum, plasma</td>
<td>Progression</td>
<td>Human</td>
<td>(82,169,170)</td>
</tr>
<tr>
<td>AChR</td>
<td>Serum</td>
<td>Unknown</td>
<td>Human</td>
<td>(60), (171)</td>
</tr>
<tr>
<td>Cellular response / infiltration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microglia activation, FcrR1 expression</td>
<td>Spinal cord</td>
<td>Progression</td>
<td>hmSOD1 mice</td>
<td>(148)</td>
</tr>
<tr>
<td>Macrophages (CD68+ / CD11b−)</td>
<td>Ventral nerve root, Sciatic nerves, NMJ</td>
<td>Pre-clinical stage progression</td>
<td>hmSOD1 mice</td>
<td>(49,50,128)</td>
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<tr>
<td>Macrophages (CD169− / CD68+ / Iba1+)</td>
<td>Peripheral nervous system</td>
<td>Progression</td>
<td>hmSOD1 mice</td>
<td>(112)</td>
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<tr>
<td>Macrophages (COX-2+)</td>
<td>Brain-cortex Spinal cord</td>
<td>Sporadic</td>
<td>Human</td>
<td>(126,172)</td>
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<tr>
<td>DCs, MCP-1, microglia, macrophages (CD68+)</td>
<td>Spinal cord</td>
<td>Progression</td>
<td>Human, hmSOD1 mice</td>
<td>(39,122)</td>
</tr>
<tr>
<td>CD4+ IL-13↑ Neutrophils↑</td>
<td>PBMC</td>
<td>Fast progression</td>
<td>Human</td>
<td>(155,173)</td>
</tr>
<tr>
<td>Suppressor / cytotoxic T cells, helper T cells</td>
<td>Corticospinal tract</td>
<td>No correlation</td>
<td>Human</td>
<td>(174)</td>
</tr>
<tr>
<td>CD4↑, CD8↑, NK cells</td>
<td>Spinal cord, PBMC</td>
<td>Progression</td>
<td>Mouse</td>
<td>(125,126,149,175)</td>
</tr>
<tr>
<td>T17↑, T17↑, monocytes↑, IL-6↑</td>
<td>CSF, PBMC</td>
<td>Progression</td>
<td>Human</td>
<td>(176)</td>
</tr>
</tbody>
</table>

Abbreviations: LRP4, lipoprotein receptor-related protein 4; hmSOD1, transgenic mice expressing human mutant SOD1; ↑, upregulation of response; ↓, downregulation of response.
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Brain Muscle

Neuromuscular junction

d) Change in expression

NOGO-A ↑

Igf-1 ↓

N-CAM, MHC-I ↑

Bax-Bcl2 ↑

C4 ↓

ATP

Co-transmitter

Activation of AChR

Antibodies to AchR ?

f) Infiltration of macrophages

MCP-1 ↑ CCR2 ↓

CD11b+CD169+, CD68+, Ib1+, MCH-II ↑

g) Antibodies against

-LRP4

-Voltage Ca++ channels

-GM1 ganglioside

Ca++ imbalance

Mitochondria permeability transition

Impaired axonal transport

Disease development

Spinal cord

Motor cells axonal loss

Expression in microglia

LCA, LFA-1, MHC-I/II, CR3/R4, FcgRI

Activated microglia

Cd11c+, CD86+, CD54+, CD169+, CD68+ ↑

Cox-2 ↑, CD40 ↑

c) ↑ Expression of pro-inflammatory factors

MCP-1, IL-6, IL-13, IL-17, IL-18, IL-23,

IL-β/α, IL-1RA,

TNF-α, TNF-αR

b2) Infiltration

CD14+, T cells

CD3+ CD40L+

CD4+, NK+, CD8+, Mast cells IL17A+ ↑

IL-8, IL-15, IL-6, TNF-α

Cytokines

IL-8, IL-15, IL-6, TNF-α

h) ↑ Antibodies in blood/CSF

-HMGB1

-Voltage Ca++ channels

-GM1 ganglioside

-Fas-R (CD95)

-Anti-fetal muscular proteins

-Vascular antigens

-Neurofilaments, Nf-L

Brain

Motor cells

axonal loss

SOD iNOS

ROS

iNOS

PGE

LTB4

Muscle

Neuromuscular junction

b1) Activated microglia

Cd11c+, CD86+, CD54+, CD169+, CD68+ ↑

Cox-2 ↑, CD40 ↑

a) ↑ Expression

in microglia

LCA, LFA-1, MHC-I/II, CR3/R4, FcgRI

NMJ destruction

Axonal degeneration and denervation

P2X7 receptor

ATP

Co-transmitter

Activation of AChR

Antibodies to AchR?