

Review article

Significance of aberrant glial cell phenotypes in pathophysiology of amyotrophic lateral sclerosis



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ABSTRACT

Amyotrophic Lateral Sclerosis (ALS) is a paradigmatic neurodegenerative disease, characterized by progressive paralysis of skeletal muscles associated with motor neuron degeneration. It is well-established that glial cells play a key role in ALS pathogenesis. In transgenic rodent models for familial ALS reactive astrocytes, microglia and oligodendrocyte precursors accumulate in the degenerating spinal cord and appear to contribute to primary motor neuron death through a non-cell autonomous pathogenic mechanism. Furthermore in rats expressing the ALS-linked SOD1^{G93A} mutation, rapid spread of paralysis coincides with emergence of neurotoxic and proliferating aberrant glia cells with an astrocyte-like phenotype (AbA cells) that are found surrounding damaged motor neurons. AbAs simultaneously express astrocytic markers GFAP, S100β and Connexin-43 along with microglial markers Iba-1, CD11b and CD163. Studies with cell cultures have shown that AbAs originate from inflammatory microglial cells that undergo phenotypic transition. Because AbAs appear only after paralysis onset and exponentially increase in parallel with disease progression, they appear to actively contribute to ALS progression. While several reviews have been published on the pathogenic role of glial cells in ALS, this review focuses on emergence and pro-inflammatory activity of AbAs as part of an increasingly complex neurodegenerative microenvironment during ALS disease development.

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1. Introduction

Amyotrophic lateral sclerosis (ALS) is considered to be a paradigmatic neurodegenerative disease leading to progressive loss of upper and lower motor neurons and to progressive paralysis and death [1]. The etiologies of the disease remain unknown for sporadic cases of ALS. The discovery of more than 100 mutations in the gene encoding antioxidant enzyme Cu/Zn superoxide dismutase-1

(SOD1) in a subset of patients with familial ALS stimulated research with transgenic animal models expressing different SOD1 mutations [2,3]. The pathological process in ALS caused by mutant SOD1 appears to be related to abnormal folding and cellular accumulation of SOD1 aggregates which in turn may somehow trigger disease. Several functional defects associated with ALS have been recognized including altered redox chemistry [4,5], mitochondria dysfunction [6] endoplasmic reticulum stress [7], excitotoxicity [8,9], and distal axonopathy [10]. Since mutant SOD1 is expressed in different cell types, damage may not be restricted to motor neurons but also may affect other cell types that may or may not directly interact with motor neurons [11–13]. This results in a situation in

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which defective cell-cell communication and disrupted cell function can be anticipated. Genetically modified mice with “non-cell autonomous” toxicity of SOD1 mutations were used to elegantly demonstrate these expected defects [14–17]. In addition, genetic excision of mutant SOD1 from astrocytes slowed later disease progression in ALS mice, but had no effect on timing of disease onset [18]. Similar results were reported when mutant SOD1 expression was silenced in microglia [17]. Taken together, studies suggest a model in which mutant SOD1 induces primary motor neuron damage and distal axonopathy making glial cells active contributors to neuronal loss, thereby driving disease progression [15].

In addition to astrocytes and microglia playing a pathogenic role in ALS, Díaz-Amarilla et al. isolated a previously unknown type of aberrant astrocyte-like cell (AbA cells) from the spinal cord of rats expressing the SOD1^{G93A} mutation [19]. Remarkably, AbAs express a distinctive pattern of astrocyte and microglial markers, they proliferate more rapidly than neonatal astrocytes and are exceptionally toxic to motor neurons grown in vitro. Furthermore, AbAs replicate most abundantly during the symptomatic phase of disease and typically are found clustered near motor neurons. This suggests a link between the emergence of such cells, motor neuron pathology and progression of paralysis characteristic in the SOD1^{G93A} ALS rat model.

While other reviews focus on the role of astrocytes and microglia in ALS [11–13,20–23], this review focuses on emergence of aberrant glial cells as additional key pathogenic players in the ALS neurodegenerative microenvironment. As in other neurodegenerative diseases, neuronal death in ALS begins as a process that spreads contiguously along brain regions in an ineluctable way. This implies that establishment of the pathogenic process involve damaged primary neurons co-existing with inflammatory glial cells that accelerate the degeneration [11,24–27]. In this context, AbAs may potentially contribute to progression of paralysis in ALS and thus be a target for specific therapeutic approaches that could halt or delay paralysis progression.

2. Aberrant astrocytes-like cells (AbAs): new players in ALS

In 2005, we proposed that glial cells could follow a differentiation shift into atypical or “aberrant” phenotypes in ALS thus allowing them to escape anti-inflammatory signals and become increasingly activated to spread disease to neighboring or distant CNS motor areas. Several subsequent reports have established that astrocytes and microglia cells expressing mutant SOD1 are directly toxic to motor neurons in rodent models as well as in ALS patients [4,12,15]. In this context, the discovery of AbAs as a distinct cell type directly associated with rapid paralysis progression in ALS rats provides a new avenue to study and understand ALS pathogenesis.

Initial isolation of AbAs was from cell cultures derived from the spinal cord of symptomatic adult transgenic SOD1^{G93A} rats of five to six months of age [19]. While cultures from non-transgenic rat cords yielded low amounts of cells that failed to reach confluence or resist subsequent passages, those from paralytic rats yielded high densities of phagocytic microglia that rapidly proliferated forming clusters of elongated flat cells resembling astrocytes. As AbAs continue to proliferate after few passages, the cultures became homogeneous monolayers of flat, fusiform to polygonal cells that could be propagated for months without undergoing replicative senescence. Interestingly, culture of spinal cord cells from symptomatic SOD1^{G93A} mice also generate increased levels of microglia cells compared with pre-symptomatic mice, but these cells do not transition into proliferating astrocyte-like cells as observed in rats [19]. This finding suggests different activation mechanisms between the two species.

Cultured AbAs express typical astrocytic markers such as GFAP, vimentin, S100 β , connexin 43 and glutamine synthase, but no expression of GLT1 glutamate transporter was found. Like neonatal astrocytes, AbAs respond to forskolin stimulation displaying processes growth and increased GFAP staining [19]. Trias et al., recently provided evidence that AbAs are generated from phagocytic microglia cells that can be obtained early after establishment of primary cultures of the symptomatic SOD1^{G93A} spinal cord [28]. When such phagocytic microglia were sorted and purified by flow cytometry using the microglial surface marker CD11b, resulting cells resume active proliferation and rapidly transdifferentiate into AbAs displaying the morphology of flat, astrocyte-like cells organized in monolayers. During the transformation period from microglia to AbAs, the expression of microglia markers largely disappear, while GFAP and S100 β expression increases suggesting a profound transformation of gene expression [28].

Remarkably, AbAs simultaneously expressing markers of both microglia and astrocytes were identified in degenerating spinal cords of SOD1^{G93A} rats [28]. These cells can typically be localized in areas surrounding dying motor neurons in the ventral horn of the spinal cord and can be identified by immunostaining for astrocytic markers such as GFAP, S100 β and Cx43 and microglia markers Iba1 and CD163 [28]. These aberrant cells simultaneously displaying both astrocytic and microglial markers are surprising because such a phenotype has not been described previously in any neurodegenerative disease. In any case, co-expression of both types of markers was reported in neoplastic glioblastoma multiform cells [29,30], a human astroglial tumor having intense accompanying inflammation [30]. Similarly, spinal AbAs could originate from a phenotypic transition of inflammatory microglia into astrocyte-like cells during the symptomatic phase of ALS in rats. Previously, other studies have shown aberrant or atypical features of microglial cells in symptomatic SOD1^{G93A} rats including microglia clusters [31] and multi-nucleated giant cells [32], further indicating major phenotypic instability of microglia in rodent models for ALS. Typically, microglia cells are classified as M1 or M2, depending on the type of inflammatory activation [33]. However, based on morphology, localization, high proliferation rate and other phenotypic features; spinal AbA cells are distinct from previously described M1 or M2 microglia [34–36]. It is still not clear whether this phenotypic transition is specific to mutant SOD1-expressing microglia or might also be observed in other chronic CNS injury conditions where phagocytic microglia are found to accumulate around dying neurons [31,37,38].

AbAs are characterized by their high rate of proliferation as evidenced by Ki67 expression and BrdU incorporation. Previously, BrdU incorporation was used to demonstrate increased cell proliferation in ALS rats of NG2-positive glial progenitor cells [13], which can potentially differentiate into astrocytes. However, AbAs do not appear to be derived from NG2 oligodendrocyte progenitors found to proliferate in the ALS spinal cord after disease onset [39,40]. AbA cells in the spinal cord of symptomatic rats are NG2-negative and have a completely different morphology as well as location compared with typical NG2 cells [19].

In addition, AbAs are closely associated with motor neuron damage since their emergence in the degenerating spinal cord is coincident with disease onset, AbA numbers sharply increase at the end stage. This suggests an active role of damaged motor neurons in inducing microglia transformation, probably through release of several cytokines and trophic factors [12,21]. As will discussed below, the correlation between AbA emergence and motor neuron loss could be indicative of an active role of AbAs in mediating motor neuron damage and also inducing transformation of surrounding microglia into an aberrant phenotype. It is presently unknown whether AbAs that are formed can play a pathogenic role in ALS patients. A few studies, however, have reported increased

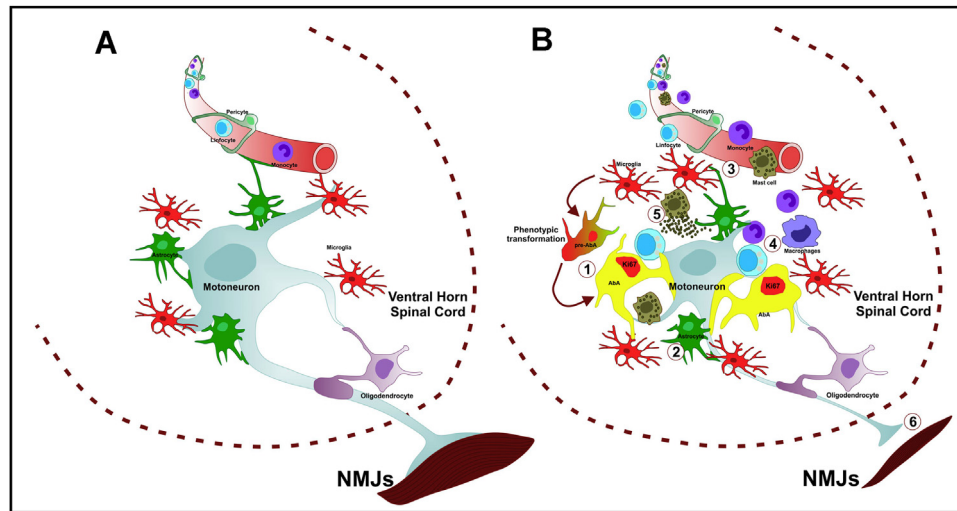


Fig. 1. Aberrant glial cells and the neurodegenerative cellular microenvironment. A) Normal constitution of the cellular microenvironment that surround motor neurons in the ventral horn of the spinal cord. B) During ALS progression different kind of cell types accumulate around damaged motor neurons. A characteristic feature is the emergence of AbAs derived from activated microglia (1). In addition, astrocytes proliferate and become reactive (2). The BBB becomes more permeable allowing the entrance of different blood cells such as monocytes, lymphocytes and mast cells (3–5). This scenario will promote a detrimental microenvironment to support motor neuron survival accelerating distal axonal degeneration, muscle denervation and paralysis progression (6).

expression of S100 proteins in astrocytes and motor neurons from ALS patients. Migheli et al. reported a population of large S100 β -positive astrocytes in the spinal cord of ALS patients, some of them typically located in close contact with motor neurons [41]. Another study of ALS patients reported a population of reactive astrocytes expressing S100A6, a protein that forms heterodimers with S100 β and could be expressed in the same cell type [42]. These results suggest that AbAs or a related type of reactive glial cell may also occur in human disease, but this still needs to be proven with more detailed studies on pathology during disease development.

3. Neurotoxic potential of aberrant glial cells

Monolayers of spinal cord astrocytes successfully support survival and differentiation of co-cultured embryonic motor neurons even in the absence of exogenous trophic factors [12,43]. The hypothesis that dysfunctional astrocytes contribute to motor neuron loss in ALS was first suggested by the observation that astrocytes briefly exposed to LPS, peroxyneutrite or FGF-1 suffer a long-lasting phenotypic transformation that cause them to induce motor neuron apoptosis [43,44]. These changes in astrocytes were likely comparable to characteristic features of astrocytosis observed in ALS cortex and spinal cord, and probably indicating that activated or inflammatory astrocytes have a decreased ability to support motor neuron survival.

The first studies reporting that astrocytes bearing the SOD1^{G93A} mutation induced apoptosis of co-cultured motor neurons showed that this toxicity was similar to that found in astrocytes activated by LPS or FGF1 [45,46]. In agreement, other laboratories also described motor neuron astrocyte toxicity and provided further evidence for the ability of mutant SOD1-expressing astrocytes to kill motor neurons derived either from mouse embryonic spinal cord or differentiated from embryonic stem cells [20,47–49]. These studies also showed that toxicity is mediated by soluble factors. Similar results were obtained with astrocytes derived from human ALS patients whether or not mutant SOD1 was expressed as reported by Haidet-Phillips et al. [50]. Taken together, these studies suggest that mutant SOD1-expressing astrocytes are not intrinsically neurotoxic but rather display phenotypic features of reactive astrocytes that result in motor neurons to become more vulnerable to stress or damage.

Interestingly, the possibility exist that astrocyte dysfunction and activation in ALS might be induced by cytokines produced by damaged motor neurons. Evidence indicates that following axotomy, motor neurons upregulate several inflammatory mediators including TNF α , FGF1, TGF β , M-CSF and nitric oxide that potentially orchestrate reactions between neighboring astrocytes and microglial [44,51–56]. In turn, chronically activated glial cells can become increasingly activated independent of neuronal signaling. In this scenario, crosstalk between different cell types in the neurodegenerative cellular microenvironment seems critical to understand ALS pathophysiology.

When compared with mutant SOD1 bearing astrocytes and microglia, AbAs appear to be the most toxic cells yet identified for motor neurons. Díaz-Amarilla et al. assessed the neurotoxic potential of AbAs on survival of embryonic motor neurons isolated by immunopanning [19,43]. In experiments where motor neurons were seeded on confluent AbA monolayers, motor neuron survival was less than 10%, suggesting that AbAs create a potentially non-permissive environment for motor neuron growth and differentiation. AbA cell-derived conditioned media was also found to be highly toxic for embryonic motor neurons. Dilutions of AbA conditioned media up to 1000-fold were able induce motor neuron death with a potency compared with that of 10-fold dilutions of conditioned media derived from neonatal astrocytes expressing SOD1^{G93A} [19,20,57]. AbAs are thus the most toxic cell type known for motor neurons in that they are very likely capable of producing active and highly neurotoxic soluble factors. The neurotoxicity of AbA conditioned media is observed only in motor neurons but not for other types of neurons suggesting that specific signaling mechanisms are involved. Although the mechanism of AbA neurotoxicity is under active investigation, the possibility exists that AbAs produce cytokines, excitotoxins or trophic factors that may specifically kill motor neurons. For instance, AbAs express high levels of the gap junction protein Cx43, which is also found in astrocytes and is known to modulate their proliferation, migration and differentiation [58]. In rodent models of ALS has been recently reported that Cx43 in SOD1 astrocytes contributes to motor neuron toxicity [59]. Cx43 can form hemichannels on the surface of cells that can potentially permit the release of toxic and inflammatory mediators such as ATP [60]. In turn, released ATP may prompt chemotaxis of microglia [61] activation of neighboring astrocytes

[62] and excitotoxic signaling to motor neurons [63]. The remarkable expression of S100 β in AbAs might also exert paracrine effects in surrounding glial cells and elicit neurotoxicity in nearby motor neurons [64]. Thus, AbAs potentially play an important role in promoting glial activation and motor neuron damage through various complex molecular mechanisms.

4. Aberrant glial cells and the neurodegenerative cellular microenvironment

Because AbAs are associated with rapid progression of paralysis in SOD1^{G93A} rats, they may be associated with increasingly detrimental inflammatory reactions in the microenvironment around motor neurons in the ventral spinal cord. As shown in Fig. 1 during ALS progression, different kind of cell types accumulate around damaged motor neurons. AbAs may facilitate the generation of this neurodegenerative cellular microenvironment that promotes rapid progression of the disease to adjacent areas of the neuraxis. As a result of AbA emergence and subsequent inflammatory reactions, the blood brain barrier (BBB) could be disrupted enabling new types of blood-born cells or precursors to enter into diseased regions. Altered permeability of the BBB has been reported in rodent models for ALS [25,65], however, the contribution of AbAs to BBB disruption remains unknown. In addition to lymphocytes and dendritic cells infiltrating the degenerating spinal cord [66,67], evidence indicate that degranulating mast cells expressing IL-17 can be observed in the spinal cord of ALS patients [68] It is assumed that even in low numbers, mast cells play an important role in triggering chronic inflammation in the CNS [69,70]. Also, resident mast cells located in the meninges containing TNF α and vasoactive mediators, may regulate permeability of the BBB in the surroundings and facilitate entry of lymphocytes and neutrophils. Thus, a potential interaction between AbAs and mast cells must be explored in new experimental settings.

Monocytes may also be attracted by AbAs to become relevant players in the ALS cellular microenvironment. In particular, Ly6C^{hi} monocytes displaying a pro-inflammatory phenotype seem to be recruited in the degenerating spinal cord of a murine model for ALS during disease progression [71]. Specific inhibition of these cell types attenuate motor neuron loss and disease progression, suggesting a contribution of blood-born inflammatory cells to ALS pathophysiology. Interestingly, a clinical trial with the drug NP001, that specifically inhibits peripheral monocytes/macrophages with inflammatory potential, was shown to be effective in a small subset of patients with ALS [72,73]. It is unknown whether these drugs targeting peripheral immune cells modify the CNS cellular microenvironment or simply modulate inflammation in motor axons and neuromuscular junction [74].

5. Conclusion

The appearance of aberrant glial cells in ALS is likely dependent on triggering of specific interactions between damaged neurons, glial cells and the subsequent remodeling in the neurodegenerative microenvironment. The concept of a 'functional unit' formed by a neuron together with the adjacent glial, endothelial and immune system cells underscores the complexity of cellular interaction in ALS pathophysiology. When motor neuron function is perturbed by expression of mutant SOD1 or another insult, glial cells can proliferate, be replaced or adopt a reactive phenotype that can largely counteract the insult and eventually prevent neuronal degeneration. However, when the regenerative glial response is altered by the emergence of aberrant glia phenotypes, such as AbAs, the intervention of these new cells will potentially change the equilibrium or homeostasis of the neuron-glia functional unit to induce overt

pathology and motor neuron loss. Not only may glial cell phenotypes vary greatly but also other new cell types may be generated from resident or peripheral precursor cells. In this context, the discovery of AbAs in rats expressing the SOD1^{G93A} mutation opens new avenues for research to find populations of other aberrant cell types having dysfunctional phenotypes and intrinsic toxicity. Remarkably, detailed characterization of such cells in triggering pathology may prompt the development as well as understanding of new therapies specifically targeting abnormal cells. In this context, AbA cells appear to be vulnerable to drugs controlling the phenotypic instability of their microglial precursors as well as the exaggerated proliferation rate. Future studies will have to elucidate whether targeting AbA cells with specific drugs can slow the paralysis progression as anticipated by our previous work.

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