Concise review: The Cellular Conspiracy of ALS

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Running head: *The Cellular Conspiracy of ALS*

Keywords: human pluripotent stem cells (hPSCs), cell-autonomous, non-cell-autonomous, disease mechanisms, *in vitro* modelling

Abstract

Amyotrophic lateral sclerosis (ALS) is incurable and devastating. A dearth of therapies has galvanized experimental focus onto the cellular and molecular mechanisms that both initiate and subsequently drive selective motor neuron (MN) degeneration. A traditional view regarding ALS pathogenesis posits that disease-specific injury to a subtype of neurons is mechanistically cell-autonomous. This "neuron-centric" view has biased past research efforts. However, a wealth of accumulating evidence now strongly implicates non-neuronal cells as being major determinants of ALS. Although animal models have proven invaluable in basic neuroscience research, a growing number of studies confirm fundamental interspecies differences between popular model organisms and the human condition. This may in part explain the failure of therapeutic translation from rodent pre-clinical models. It follows that integration of a human experimental platform utilizing patient-specific induced pluripotent stem cells (hiPSCs) may be necessary to capture the complexity of human neurodegeneration with more fidelity. Integration of enriched human neuronal and glial experimental platforms into the existing repertoire of preclinical models might prove transformational for clinical trial outcomes in neurodegeneration. Such reductionist and integrated cross-modal approaches allow systematic elucidation of cell-autonomous and non-cell-autonomous mechanisms of disease, which may then provide novel cellular targets for therapeutic intervention.

Introduction

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive and universally fatal condition, which leads to selective motor neuron (MN) degeneration [1]. ALS cases are predominantly sporadic (sALS) but approximately 10% are familial (fALS), the two being indistinguishable on clinical examination. Historically the first mutations linked to fALS were identified in the superoxide dismutase 1 (SOD1) gene in 1993 [2], followed by an almost decade-long hiatus before a second phase of productive genetic discovery. Indeed, more than 20 different genes have now been linked to ALS, including relatively common mutations in chromosome 9 open reading frame 72 *(C9ORF72)* [3] and rarer mutations in TAR DNA Binding Protein *(TARDBP)* [4], Fused in Sarcoma/Translocated in Sarcoma *(FUS)* [5] and Valosin Containing Protein *(VCP)* [6], among several others. Until recently, studies examining the interplay between human neurons and glia in neurodegeneration have been hampered by relative inaccessibility to these cellular populations. The advent of hiPSCs through reprogramming technology, together with directed differentiation techniques, have greatly increased access, allowing researchers to obtain - *a la carte* – enriched populations of specific neuronal and glial cell types [7-9]. In order to harness the full potential of hiPSC models, an understanding of developmental principles underpinning the generation of neuronal and glial diversity is a prerequisite. Studies using this platform for disease modeling have largely focused on cell-autonomous mechanisms of neuronal degeneration, leaving the role of glia in this context comparatively understudied.

Cellular interplay in the central nervous system (CNS) is spatio-temporally regulated in both development and disease. The major cellular contributors include a diverse range of region-specific neuronal subtypes in addition to both macro- and microglia (MG). Neurons and macroglia, astrocytes (ACs) and oligodendrocytes, are developmentally ectodermal in origin, while microglia arise from the mesoderm (yolk-sac). CNS cells exhibit both homotypic and heterotypic interactions, and show profound region-specific functional heterogeneity. Glia and neurons in the CNS interact with one another through direct contact (e.g. gap junctions or receptor-mediated), secreted factors (e.g. molecules or exosomes) or a combination of these, which are crucial to establish

and refine functional structures, including the tripartite synapse. Given such intricate connectivity, it seems eminently reasonable – if not likely - that glial cells would play key roles in the majority of neurological diseases. Indeed, cell-autonomous and non-cell-autonomous glial mechanisms of disease are now increasingly being implicated as playing diverse and pivotal roles in human ageing and neurodegeneration [10].

Glia as pertetrators of ALS

A 'neuron-centric' theoretical construct of ALS has been increasingly challenged over the last decade or two. The mechanisms by which glia can exert deleterious effects on neighboring neurons include i) through failure of support or homeostatic function, ii) toxic gain of function and release of toxic substances or iii) a combination of these mechanisms. Such effects can be contact-dependent and/or operate through a soluble factor (see Figure 1). Temporal aspects are also important to consider in this context; i.e. acute vs chronic activation of glial cells may well determine how effective their responses are in ALS and it is possible that chronic activation states eventually become maladaptive and adversely affect MNs. Assays to investigate cell-cell interactions can be experimentally operationalized through a variety of approaches, including i) co-seeding different cell types, ii) "sandwich" cultures where two established monolayers are brought into close proximity, iii) transwell-based co-culture, iv) microfluidic devices and v) conditioned medium transfer. These approaches with their respective strengths and weaknesses have been recently reviewed elsewhere [11]. The choice of paradigm depends on whether the experiment aims to discriminate contact-dependent from contact-independent mechanisms of non-cell-autonomous injury. An important consideration here is the existence of cell-autonomous glial pathology, which remains relatively understudied in ALS and may indeed contribute to the failure of supportive capacity. Illuminating non-neuronal mechanisms of disease raises the important prospect of designing innovative therapeutic approaches that target glial cells. Further relevant and open questions in the field are presented in Box 1. We now discuss 2 exemplar cell types implicated in mouse models of ALS: MG and ACs.

Lessons from rodent models of ALS

Microglia-motor neuron dialogue: The role of MG as a non-cell autonomous driver in MN degeneration has been studied in different experimental platforms, and a number of mechanisms proposed (Figure 1). Important mouse-chimera studies using lineage-specific expression of mutant SOD-1 first demonstrated the role of MNs in disease onset and early progression, while implicating MG as key drivers of late-phase disease progression [12]. Moreover, a subsequent study found that significant alterations in microglial populations are present in SOD-1 models at pre-symptomatic stages, with a decrease in microglia before onset and the emergence of two distinct populations after symptom manifestation [13]. Of the several specific MG mechanisms that have been proposed, one study found significantly increased levels of the oncoprotein c-RET in activated microglia in $SOD1^{G93A}$ mice. The increase was cell-type specific (MG>MN), age-dependent, and proposed to non-cell-autonomously impair GDNF signaling in MNs [14]. More recent experiments have shown that expression of mutant SOD-1 significantly increases MG secretion of neurotoxic cytokines [15] and that perturbing pro-inflammatory MG activation through deletion of NF-κB – a master regulator of inflammation - rescues MNs from MG-mediated death in models of ALS [16]. Indeed a micro-RNA (miR-125b) has been found to play a salient role in microglial activation through direct repression of ubiquitin-editing enzyme A20, a potent suppressor of the NF-κB pathway [17]. Further evidence implicating MG in the most common genetic form of ALS comes from mice lacking the *C9orf72* ortholog in all tissues. Among other immune-related phenotypes, perturbed immune responses in microglia were uncovered [18]. Following earlier confirmation of TDP-43 in the cerebrospinal fluid of patients with ALS [19], a recent study also demonstrated that extracellular TDP-43 aggregates can trigger activation of the NF-kB pathway in MG, invoking secretion of IL-1b and IL-18 [20]. Interestingly, a MG-specific inducible conditional TDP-43 knockout mouse line resulted in significant synaptic loss, while enhancing amyloid clearance [21]. The importance of MG in ALS pathogenesis is further reinforced by the finding of an early innate immune response in the motor cortex of ALS $SOD1^{G93A}$ mice [22].

Astrocyte-motor neuron dialogue: Several studies have implicated ACs in the pathobiology of ALS through a number of mechanisms, including both active and secondary toxicity (Figure 2). An early study addressing this issue reported that restricted expression of $SOD1^{G86R}$ in ACs causes astrocytosis but not MN degeneration [23]. However, ex-vivo $SOD1^{G93A}$ ACs have subsequently been used in physical co-culture and AC conditioned medium (ACM) experiments, confirming that they are toxic to both primary and mouse embryonic stem cell (mESC)-derived MNs [24, 25]. Nagai and coworkers demonstrated that both toxicity and vulnerability are cell-type specific as only mutant ACs (not fibroblasts or other cell types) produced non-cell-autonomous toxicity. Additionally this effect was specifically directed towards MNs, while other neuronal subtypes including spinal GABAergic, dorsal root ganglion or mESC-derived interneurons, were not affected. These aforementioned studies have established that toxic effects are mediated through a soluble factor and a BAX-dependent mechanism [24, 25]. Di Giorgio and coworkers then confirmed that the same SOD1 non-cell-autonomous toxicity was evident when performing co-cultures with hESC-derived MNs [26].

A number of alternative possible mechanisms exist through which AC-mediated MN injury can occur, either through loss of supportive function or active toxicity. These include perturbations in: expression of AC receptors / transporters, release or metabolism of AC transmitters, synthesis and release of chemokines, cytokines, and free radical generation or coupling of gap junctions (Figure 1B). $SOD1^{G93A}$ astrocytes were found to be a key driver of disease progression in an ALS model [27]. Indeed, transplanted ACs expressing mutant SOD1 induce MN degeneration in wild-type rats [28]. Particularly in SOD1 models, active AC toxicity to MNs has been observed, mediated by production of reactive oxygen species, which has been shown to induce MN hyper-excitability [29, 30] and MN degeneration through mitochondrial dysfunction in ACs [31]. Another proposed mechanism for direct non-cell autonomous toxicity to MNs is AC-mediated perturbation of MN autophagy, exacerbating underlying cell-autonomous toxicity by decreasing MN ability to handle accumulating misfolded proteins [32, 33].

Although relatively understudied, a number of non-cell autonomous mechanisms derive from loss of supportive capacity rather than active toxicity. For example, $SOD1^{G93A}$ fALS ACs induce MN death at least in part through reducing metabolic support (lactate release) and activating pro-nerve growth factor-p75 receptor signaling pathway at the expense of mature nerve growth factor production [34]. Converging lines of evidence suggest that regulation of the AC glutamate transporter EAAT2 in ALS non-cell-autonomously determines MN survival. The intuitive mechanism here is excitotoxicity through failure of AC glutamate clearance [35], although one study has suggested that sumoylated carboxy-terminal fragments of EAAT2 accumulate in the AC nucleus and cause impaired axonal growth in co-cultured MNs independently of excitotoxicity [36]. Another example of loss of AC support is the disrupted GluR2-regulating capacity of mutant SOD1 ACs, which renders MNs vulnerable to excitotoxicty [37]. A BCL-2 family protein called Bid is elevated in $SOD1^{G93A}$ ACs and acts as a key regulator for activating NF-κB [38]. In contrast to MG, selective NF-κB inhibition in ACs is not sufficient to rescue MN death [16]. NLRP3 'inflammasome' complexes are crucial for the processing and release of IL1b and IL18, and are predominantly expressed in ACs [39]. To add further complexity, a regionally determined functional heterogeneity of ACs [40] might underlie region-specific responses to the same mode of injury. Furthermore, different phases of the same disease may invoke diverse responses in ACs.

Beyond the glial-neuronal dialogue: Lineage specific translational profiling in a $SOD1^{G37R}$ model has revealed sequential changes first in MNs (ER stress, synapse and metabolic changes) followed by ACs (abnormal inflammatory responses and metabolism) and then oligodendrocytes (membrane and lipid signaling defects) reinforcing the concept of cell type-specific contributions to different phases of disease [41]. Indeed stereotyped intercellular interactions are likely also perturbed in a disease stage-specific manner (Figure 3). In vivo, diminished AC $SOD1^{G93A}$ expression, in addition to slowing disease progression, also delayed MG activation [27]. Conversely, transplantation of $SOD1^{G93A}$ ACs into wild-type rats caused MN degeneration, at least in part, through MG activation [28]. Indeed, AC activation by ALS MNs stimulates secretion of lipocalin (lcn2) in rats, which in turn orchestrates and amplifies various downstream effects through

actions on quiescent ACs, MG and MNs [42]. Subsequent studies have suggested that AC-derived TGF-β1 accelerates disease progression in vivo by interfering with the neuroprotective functions of MG and T cells [43]. MG-AC signaling can also lead to the acquisition of toxic functions in ACs. Specifically, a recent study demonstrated that by secreting Il-1α, TNF and C1q, activated microglia induce a toxic AC phenotype, which impairs their ability to promote neuronal survival, synaptogenesis and phagocytosis [44]. An intriguing set of experiments has also raised the possibility of cell fate transition from MG into AC-like cells, which coincides with disease onset in a model of inherited ALS [45]. Clearly there will exist myriad cellular mechanisms of disease beyond MNs, MG and ACs. Indeed there is an early but evolving literature in ALS implicating several other neuronal subtypes (e.g. interneurons [46, 47]), glia (e.g. oligodendrocytes [48, 49]) and immune cells including CD4+ regulatory T cells, cytotoxic CD8+ T cells and natural killer cells [50-52].

Limitations of animal ALS models: From the selected examples above, it is clear that animal models are indispensible and have provided invaluable insight into issues of cellular autonomy in ALS. It is noteworthy, however, that the vast majority of studies have been performed in overexpression SOD1 models, which do not convey mutant proteins at pathophysiological levels. Crucially, SOD1 mutations do not exhibit the major pathological hallmark of TDP-43 proteinopathy as observed in >97% of human ALS cases [53]. This pathological difference reinforces the importance of validating findings from SOD1 models in other experimental platforms that recapitulate this hallmark feature. More broadly, there has been an overwhelming failure of translation from animal pre-clinical models of neurodegeneration to impactful clinical therapies, possibly reflecting underlying interspecies differences. Many clear evolutionary differences exist between mice and man, including at gross neuroanatomical, circuit, cellular and molecular levels [54]. To capture the complexity of the human clinical disease state with precision, it seems imperative to complement existing animal-based approaches with human experimental pre-clinical models, such as hiPSCs but also with postmortem tissue. We will now focus on how human platforms can help to directly elucidate issues of cellular autonomy in

neurodegeneration (see Box 2), while the wider relevance of hiPSCs in regenerative neurology has recently been reviewed elsewhere [55].

Delineating cellular autonomy in human ALS

Human post-mortem tissue: laser capture microdissection of human post-mortem tissue seems an attractive approach to deconvoluting cell type-specific contributions in neurological disease. However, this method introduces bias towards the cellular soma and away from the axon and dendrites (which are less efficiently captured). Likewise, the neuropil often 'contaminates' attempts to isolate glial cells using this approach. A large selection of post-mortem samples across different age groups raises the prospect of uncovering age-dependent changes in the human brain, which represents a crucial risk factor for neurodegeneration. Indeed bioinformatic approaches have recently been successfully utilized to deconvolute cell-type specific transcriptional signatures upon ageing from microarray-based studies of heterogeneous postmortem tissue [56]. It is also possible to 'immunopan' specific cell types from postmortem tissue, as was recently performed across a diverse age range to characterize temporal changes in the AC transcriptome [57]. A similar study conducted transcriptome-wide analyses on purified MG from mouse and human tissue and reported a divergence in age-related expression patterns, further reinforcing the importance of validating key experimental findings with human samples [58]. Human post mortem tissue additionally permits ex-vivo culture of certain cell types, which has proved a valuable resource for studies of human AC-mediated non-cell-autonomous injury [59, 60] (discussed further below). An important consideration of human postmortem tissue is that it represents a late stage of disease, and cannot therefore effectively inform on early pathogenic events. Indeed, even in cases obtained early in their disease course, it is important to recognize that at a molecular and cellular level, the disease processes are underway years before clinical symptoms appear and even these cases will therefore not allow elucidation of early molecular pathogenic events. The use of post-mortem tissue is further complicated by varying post-mortem intervals of delay, which can introduce significant variability between studies, but nevertheless represents a crucial approach in understanding cellular autonomy

in neurodegeneration (particularly when integrated with the other experimental model systems discussed in this review).

HiPSCs: the state-of-the-art. The iPSC platform offers the ability to direct differentiation to any human lineage in an ontogeny-recapitulating manner. A fundamental prerequisite to establishing iPSC models is therefore a robust understanding of the developmental programmes of morphogenetic cues required to direct differentiation to regionally specified neuronal and glial subtypes; as recently reviewed elsewhere [10, 61-64]. This presents the experimental opportunity to resolve molecular mechanisms that underlie distinct stages of lineage restriction to different neuronal and glial subtypes by faithfully recapitulating human neurodevelopment [65]. This is a crucial advantage as a recognized phase of compensated dysfunction occurs prior to clinical manifestation of neurodegenerative disease. Furthermore, patient-specific iPSCs convey mutations at pathophysiological levels (or the underlying genetic complement in sporadic cases). These attributes together make hiPSCs a powerful experimental tool capable of deconvoluting the complexity of heterologous cell-cell interactions in health and disease.

Where underlying developmental programmes are well understood and defined neuronal subtypes can be specified, hiPSC biology has led to a step change in the discovery of cellular and molecular phenotypes or therapeutic potential [66-74]. Elucidation of cell-autonomous mechanisms of disease is eminently achievable through enriched monoculture of a particular cell type. Indeed, generating regionally distinct populations of neurons (e.g. spinal motor neurons and cortical neurons) can help to discriminate neuronal subtype vulnerability [75] or identify shared mechanisms in multi-regional disorders (e.g. the spectrum of ALS and frontotemporal lobar dementia [76]). Researchers can build on these early phenotyping studies by probing neuronal or glial monoculture responses to physiological stimuli [77, 78] or by incrementally adding cellular complexity through co-culture paradigms [79]. These approaches permit deeper understanding of non-cell-autonomous mechanisms of disease as they combine some of the complexity of in vivo circuitry with the ability to 'control' the in vitro system. Human iPSC-based models also allow fully human co-culture paradigms, which addresses concern over species-specific aspects of intercellular

communication. Techniques such as high-content imaging (HCi) have provided crucial insights into cellular vulnerability by increased assay sensitivity when compared with traditional cross-sectional imaging methods [79-82]. However, one of the main rate-limiting factors has been elucidating the developmental programme of morphogenetic cues to direct differentiation to regionalized subtypes of neurons in high enough enrichment. For example, studies systematically examining upper MNs (i.e. cortical layer V) in ALS using hiPSCs are lacking. This is largely due to the difficulty in specifying distinct cortical layers reproducibly and in an enriched manner. The lack of reliable cell surface markers for cortical MNs further compounds this problem.

AC-mediated non-cell-autonomous mechanisms of disease that were first described in ALS animal models over the last decade or so, have been broadly validated and extended in a variety of human models (hiPSCs and ex-vivo cultures derived from port-mortem cases); representative studies are summarized in table 1. Evidence thus far points clearly to release of toxic substances by ACs, although failure of supportive mechanisms remains relatively understudied in the human context. Importantly, the hiPSC platform also allows dissection of early AC cell autonomous effects in ALS [78-80], which adds an additional layer of complexity to the intricate cellular interplay. AC survival defects are often difficult to capture through traditional cross-sectional analysis due to the fact that astrocytes retain proliferative capacity, thus reinforcing the utility of a HCi approach [82]. Another crucial aspect to consider is the level of maturation and functional activation achievable in glial cultures derived from patient-specific iPSCs. Although iPSC-AC populations have been functionally characterized in vitro [80, 83, 84], a comprehensive functional comparison with in vivo ACs has only been attempted recently in one elegant study [85]. Few studies have attempted to define basal human iPSC-derived AC reactivity state, and how this might be manipulated (i.e. to induce quiescence or reactivity) in order to examine their effects in different disease paradigms [78, 86, 87]. Increasing recognition of region-specific functional heterogeneity of ACs [40, 88, 89] also argues for studies focusing on their regional specification from hiPSCs; indeed spinal and cortical AC specification has already been accomplished [84]. Directed differentiation of MG from hiPSCs has also been achieved [90], and this

will be an important tool to resolve cell-autonomous and non-cell-autonomous effects of human MG in ALS, following on from earlier rodent studies summarized above.

It remains unresolved how early molecular disturbances begin and the hypothesis that adult-onset neurogenetic disorders may be manifest at molecular and cellular levels during neurodevelopment is supported by the numerous hiPSC studies demonstrating clear phenotypes in what is essentially a developmental system (reviewed in [10, 55]). Indeed, existing evidence suggests that hiPSC derivatives upon terminal differentiation resemble a fetal maturational state [67, 91, 92]. One strategy to 'preserve' the age of the donor cell is to bypass induction of pluripotency and directly 'transdifferentiate' fibroblasts into a target neuronal or glial population [93], although this approach has the disadvantage of limited expansion of the target population. Recent studies have also pharmacologically induced ageing using the telomerase inhibitor 2-[(E)-3-naphtha- len-2-yl-but-2-enoylamino]-benzoic acid (BIBR1532) [94] or expression of Progerin, a truncated form of lamin A that is associated with premature aging [67]. In other cases researchers have successfully cultured cells over a protracted period of time to uncover age-related phenotypes [95]. Cellular ageing in vitro is likely to result from a complex interplay of different factors and gene expression programs [92, 93]. Future protocols to induce cellular ageing in vitro are likely to use a combinatorial approach, including co-culture with non-neuronal cell types. Clearly the *in vivo* environment may readily compensate such initiating molecular perturbations but their identification is crucial in guiding mechanistically targeted therapies. Furthermore, delineation of culprit cell types and how they conspire in neurodegeneration will clearly be of paramount importance in considering therapeutic strategy. Given the developmental nature of the hiPSC paradigm, the stability of cellular identity is also worthy of consideration, particularly in the setting of co-cultures where either cellular component may encounter a range of unfamiliar extrinsic cues.

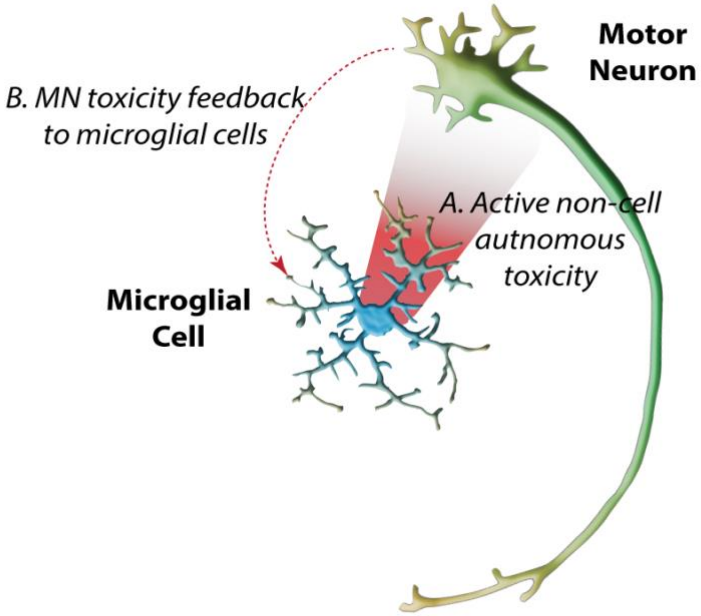
Another noteworthy limitation of hiPSC studies so far is the lack of ordered architecture in cell-cell interactions. While neurons and glia in vivo interact within the context of organized circuitry (e.g. upper MN to lower MN to muscle), most hiPSC in vitro models lack the ability to organize into a cellular

configuration that resembles the original circuit architecture in vivo due to facile culture substrates used. This can potentially be overcome by exploiting the interface between stem cell technology and bioengineering approaches to impose basic characteristics like directionality to approximate physiologically functional circuits. Introducing 3D culture systems to hiPSC modeling is also noteworthy here. Several "organ-on-chip" systems have been developed specifically for recapitulating aspects of neurodevelopment in vitro, including: (i) bioengineering solutions to physically guide axonal extension while segregating different cell types in different chambers to evaluate drug-treatments and cellular interactions [96-98], in some cases permitting direct recording of neuronal activity [99]; (ii) co-culture of different neuronal populations to create complex networks but in an organized fashion, resembling in vivo neuronal circuitry [100]; (iii) generating 3D structures form hiPSC-derived neural precursors, either using artificial scaffolds [101-103] or creating brain 'organoids' [104-106]. Combining these approaches allows for a more complex culture system in vitro, permitting multi-lineage disease modeling. Recent examples relevant to ALS include bioengineered neuromuscular junctions in vitro [107, 108]. These approaches extend disease modeling beyond enriched monoculture of an individual cell type to recapitulation of neuronal circuits, which will in turn evolve to multilineage co-cultures permitting insight into the cascade of cellular and molecular events that initiate disease and underlie progression.

Summary

To systematically gain insight into the cellular interplay underlying neurodegeneration, it is crucial to first elucidate cell-autonomous phenotypes in monoculture. This then serves as a useful reference for co-culture experiments. Additionally, discerning whether specific mechanisms of non-cell-autonomous injury require cell-cell contact or are diffusible in nature will help to inform optimal approaches to therapy development. To complement this human in-vitro modeling approach, in-vivo studies can provide crucial insight into the sequence of cellular involvement in a specific disease. Likewise, human postmortem tissue can then provide useful information about later stages of a disease process by correlating neuropathology with the clinical history [109-113]. It is important to

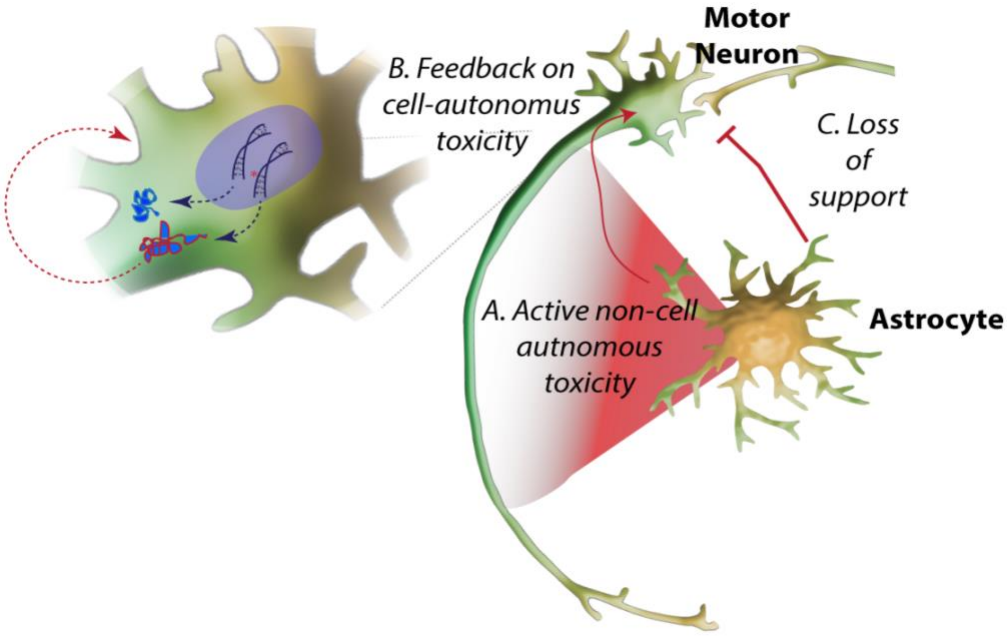
recognize that each disease model alone does not capture the full complexity of human pathophysiology, and so integrating human and animal experimental models with human post-mortem tissue work is a key step to driving high-confidence cross-modal discovery science. The overarching purpose of uncovering cell-autonomous and non-cell-autonomous mechanisms of disease is to identify which processes within (and between) neurons and glia represent crucial 'tipping' points from a state of compensated dysfunction to irreversible decompensation and neurodegeneration. This approach will help to prioritise therapeutic efforts around these specific salient events, which will likely have real impact on slowing down, stopping or ultimately reversing human ALS.



Microglia Non-Cell Autonomus Toxicity Mechanisms

Gain of Toxic Function	
Increased c-RET levels impairing GDNF signaling in MNs	*Ryu et al 2011*
Secretion of other toxic factors (yet unidentified)	*Roberts et al 2013*
Secretion of inflamatory factors (e.g. TNFα)	*Liu et al 2009, Frakes et al 2014, Leal-Lasarte et al. 2017*
Loss/Dysregulation of Function	
Decreased number and altered populations	*Gerber et al 2012*
Secondary effect of microglial cell-autonomous toxocity	*O'Rourke et al 2016*

Figure 1. Microglial non-cell autonomous toxicity. A number of non-cell autonomous mechanisms of toxicity have been observed in MG-MN interactions, placing MG as important players in driving pathology. Active toxicity mechanisms (A) include increased levels of c-RET, secretion of pro-inflammatory factors and direct toxicity via secretion of yet unidentified mediators. MG dysfunction has also been observed as a consequence of exposure to stress response factors produced by cell-autonomous MN toxicity mechanisms (B).



Astrocytes Non-Cell Autonomus Toxicity Mechanisms

Gain of Toxic Function	
ROS production inducing hyperxitability	*Fritz et al 2014, Rojas et al 2015*
Secretion of mutant proteins (SOD1)	*Zhao et al 2009*
Inhibition of autophagy in MNs	*Tripathi et al. 2017 , Madil et al. 2017*
Secondary active toxicity (e.g. mitochindrial dysfunction)	*Cassina et al 2008*
Loss of Support Function	
Excitotoxicity (Reduced EAAT2)	*Ganel et al 2006, Foran et al 2011*
Failure to regulate MN vulnerability (GluR2)	*Van Damme et al 2009*
Cell-autonomous glial toxicity	*Hall et al 2017, Serio et al 2013*
Failure of glia-neuronal metabolic crosstalk	*Ferraiouolo et al 2011*

Figure 2. Astrocyte non-cell autonomous toxicity. AC non-cell autonomous toxicity to MNs has been demonstrated in a wide range of models and definitely has a central role in disease progression. Several direct gain of toxic function mechanisms have been described (A) including: production of ROS, secretion of mutant proteins (e.g. SOD1) in the extracellular space, or other secondary dysfunction. ACs can also inhibit key coping mechanisms that exacerbate underlying MN cell-autonomous toxicity, for example autophagy. A range of putative loss of functional mechanisms have been reported (C), including reduced EAAT2 expression or failure to regulate GluR2 expression in MNs, both leading to excitotoxicity. Similarly depletion of functional AC populations due to cell autonomous glial toxicity is noteworthy here.

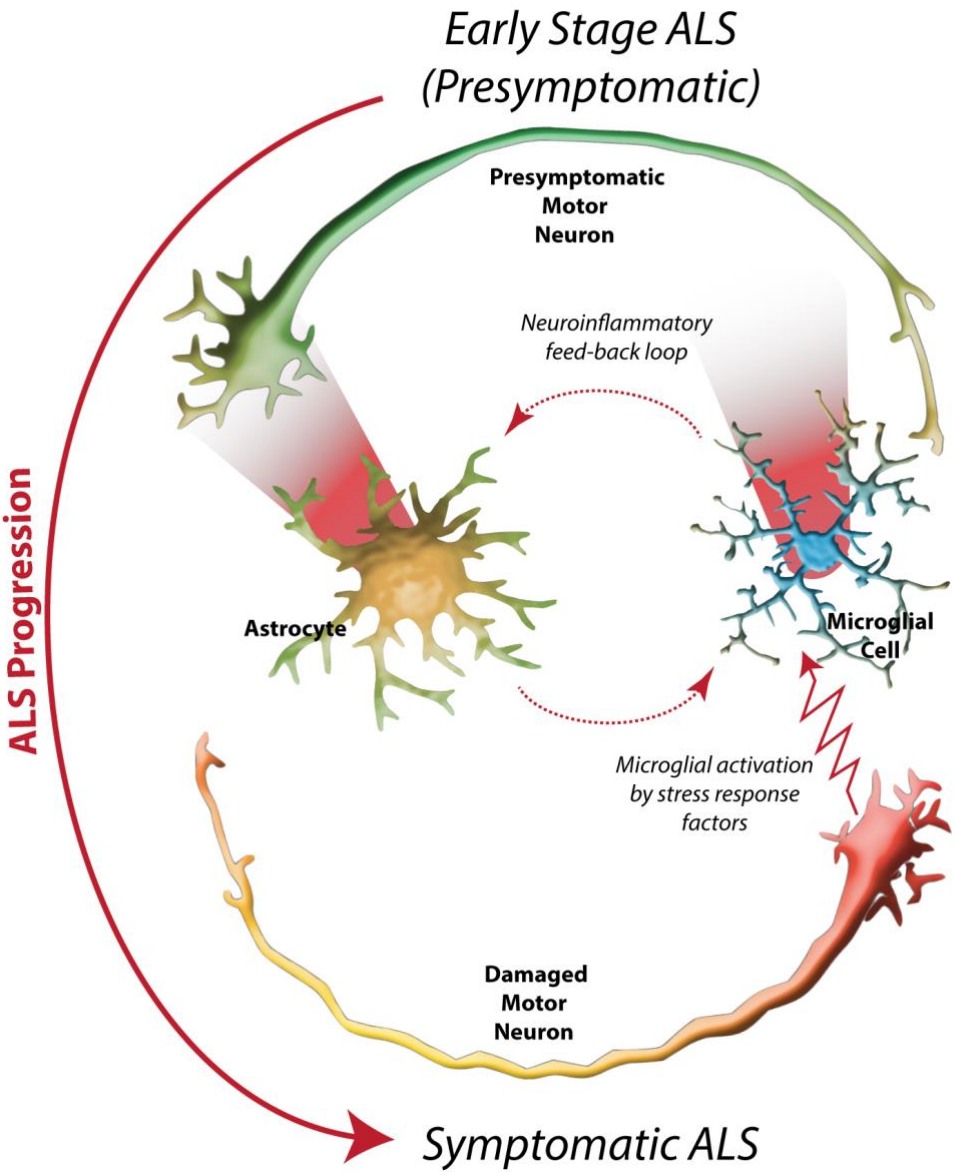


Figure 3. A cycle of cellular autonomy in ALS pathobiology. The complex interplay between MNs, AC and MG changes during different phases of disease and the non-cell autonomous effects of glial cells can be both causes and consequences of cell-autonomous toxicity in MNs. For example, during the early phases of disease, cell-autonomous toxicity and non-cell autonomous active toxicity from ACs causes damage to the MN populations and release of stress response factors and mutant proteins; this in turn acts as a trigger for the activation of a neuro-inflammatory response in MG cells, which then become more toxic to the MN population.

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