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Inflammation and neurovascular changes in amyotrophic lateral sclerosis

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ABSTRACT

Neuroinflammation in now established as an important factor in the pathogenesis of many neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). At various time points, astrocytes and microglia are markedly activated, either producing neuroprotective or pro-inflammatory molecules, which can decrease or increase the rate of primary motor neuron degeneration respectively. Recent research has shown that this neuroinflammatory component is affected by the peripheral immune system; T lymphocytes in particular are able to cross into the brain and spinal cord parenchyma, where they interact with resident microglia, either inducing them to adopt an M1 (cytotoxic) or M2 (protective) phenotype, depending on the stage of disease. Clearly understanding the changes that occur to allow the interaction between peripheral and central immune responses will be essential in any attempt to manipulate the disease process via neuroinflammatory mechanisms. However, our understanding of the endothelial changes, which facilitate the infiltration of peripheral immune cells into the brain and spinal cord, is still in its infancy. There are suggestions, though, of up-regulation of cellular adhesion molecules, which are able to arrest circulating leukocytes and facilitate diapedesis into the brain parenchyma. In addition, tight junction proteins appear to be down-regulated, leading to an increase in vascular permeability, an effect that is amplified by vascular damage late in the disease process. This review summarises our current knowledge regarding neuroinflammation, peripheral immune involvement, and endothelial changes in ALS. This article is part of a Special Issue entitled 'Neuroinflammation in neurodegeneration and neurodysfunction'.

Introduction

Amyotrophic lateral sclerosis (ALS), also known as Charcot's or Lou Gehrig's disease, is the most prevalent type of motor neuron disease (MND), affecting around 4–6 per 100,000, which corresponds to around 5,000 people in the UK. There is currently no effective disease-modifying treatment other than Riluzole, which only has a modest effect on survival (Miller, 2003; Miller et al., 2003). An improved understanding of the pathophysiology of ALS has potential for novel and more effective therapeutic intervention.

ALS is characterised by a progressive dysfunction and degeneration of both upper motor neurons comprising the corticospinal tract, and lower motor neurons arising from the brainstem nuclei and ventral roots of the spinal cord. A number of converging disease mechanisms have been proposed so far, operating either singly or in combination. These include: protein aggregation; increases in reactive oxygen species (ROS), glutamate or intracellular calcium; and mitochondrial degeneration (Keller et al., 1997; Rothstein, 2009; Viner et al., 1996). Neuroinflammation is now established as an important aspect of pathology in ALS (McGeer and McGeer, 2002; Philips and Robberecht, 2011; Weydt and Moller, 2005). Indeed, analysis of CSF from ALS patients has shown dysregulation of a number of pro- and anti-inflammatory cytokines, and growth factors, including IL-6, IL-10 GM-CSF, VEGF and IFN-γ (Mitchell et al., 2009). There is a marked activation or proliferation of both microglia and astrocytes at specific disease stages in mouse models of ALS (Hall et al., 1998; Weydt et al., 2002), which has also been shown in humans *in vivo* (Turner et al., 2004) as well as *post mortem* (Anneser et al., 2004).

Abbreviations: ALS, amyotrophic lateral sclerosis; BBB, blood–brain barrier; BDNF, brain-derived neurotrophic factor; bFGF, basic fibroblast growth factor; BSCB, blood-spinal cord barrier; CAM, cellular adhesion molecule; CD, cluster of differentiation; CNS, central nervous system; Cox2, cyclooxygenase; CSF, cerebrospinal fluid; EM, electron microscopy; GDNF, glial-derived neurotrophic factor; Gd-DTPA, gadolinium diethylenetriaminepentacetate; GLT-1, glutamate transporter-1; Glu, Glutamate; Glut-1, glucose transporter-1; GM-CSF, granulocyte macrophage colony-stimulating factor; HRE, hypoxia response element; ICAM-, 1 intercellular adhesion molecule-1; IFN-γ, interferon-γ; IGF-1, insulin-like growth factor-1; IL, interleukin; LFA-1, lymphocyte function-associated antigen; LIF, leukaemia inhibitory factor; MCP-1, monocyte chemoattractant protein-1; LPS, lipopolysaccharide; MelCAM, melanoma cellular adhesion molecule; MND, motor neuron disease; MRI, magnetic resonance imaging; mSOD1, mutant SOD1; NGF, nerve growth factor; PECAM, platelet endothelial cellular adhesion molecule; p-gp, p glycoprotein; pro-NGF, NGF precursor; ROS, reactive oxygen species; SLe^x, sialyl Lewis x; SOD1, superoxide dismutase 1; SPECT, single-photon emission computed tomography; Teff, effector T cell; TGF-β, transforming growth factor-β; TLR, toll-like receptor; TNF-α, tumour necrosis factor-α; TNF-R, tumour necrosis factor receptor; Treg, regulatory T cell; TTX, tetanus toxin; VCAM-1, vascular cellular adhesion molecule-1; VEGF, vascular endothelial growth factor; VLA-4, very late antigen-4; ZO-1, zonula occludens-1.

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Both CD4+ (helper/inducer) and CD8+ (cytotoxic) T lymphocytes have been shown to infiltrate the brains of ALS patients (Kawamata et al., 1992), and work in animal models has shown that these may be involved in complex interactions with glia that can either lead to a neuroprotective or cytotoxic phenotype (Beers et al., 2008; Chiu et al., 2008). An understanding of how, and when, lymphocytes infiltrate the CNS, and the effects that they have on resident glial cells could potentially elucidate new therapeutic strategies based on manipulating the immunological response.

The most studied model of ALS is the transgenic superoxide dismutase-1 (*SOD1*) mouse, which over-expresses a missense mutation. Different strains have been made with various mutations, each showing variable age of disease onset and speed of progression (Gurney, 1994; Ripps et al., 1995; Wong et al., 1995). Given that this model has been used in the majority of preclinical studies, particularly the $SOD1^{G93A}$ mutation, this body of work will form the basis for the review, with data from clinical studies used where appropriate. It is also important to note that, unlike many models of Alzheimer's disease (Chui et al., 1999; Games et al., 1995), the SOD1 model actually displays neuronal cell death in a manner that is consistent with the profile of cell death that is seen in the human disease. As such this progressive, degenerative model provides a useful and relevant tool for investigating the impact of neuronal cell death on the host immune system, and vice versa. Thus in a similar manner to mouse prion disease (Broom et al., 2007), the SOD1 mutant also provides a way to investigate processes that are common to a wider array of CNS pathologies.

Circulating lymphocytes can bind to the CAMs expressed of the luminal wall of CNS blood vessels, which allow them to roll, bind, and eventually migrate into adjacent CNS tissue. On entering the CNS, leukocytes can communicate directly or indirectly with astrocytes, microglia or neurons. In addition these other cell types communicate with one another via complex signalling cascades, and can also regulate the expression of the endothelium by diffusible factors.

Astrocytes

One potential source of both pro- and anti-inflammatory cytokines within the CNS is the astrocyte. Anatomically in close proximity with the BBB and BSCB, the astrocyte is ideally placed to translate signals from the periphery to the CNS. Loss of astrocytes in models of immune mediated CNS disease such as MS can have devastating consequences (Toft-Hansen et al., 2011). The downstream signalling pathways after activation are mediated by NFκB (Brambilla et al., 2005) and the cross-talk between microglia and astrocytes is important in terms of generating not only a central inflammatory response, but also in potentiating a parenchymal invasion of peripheral immune cells. In addition, astrocytes are an important component of the blood–brain barrier (BBB), and *in vitro* studies suggest that astrocytes are capable of releasing a number of inducing factors that can act on endothelial cells, such as TGF-β, GDNF and bFGF (Igarashi et al., 1999; Lee et al., 2003). Similarly, LIF can be released by endothelial cells and induce differentiation *in vitro* (Mi et al., 2001), which may continue to be important beyond development. However, the role of endothelial–astrocyte interactions remains to be clarified fully in ALS.

Whilst human ALS is clinically characterised as a disease selectively affecting upper and lower motor neurons, numerous studies in the transgenic *SOD1* mouse have illustrated that the pathology in this condition is non-cell autonomous. In other words, degeneration and death of motor neurons, and associated neurological symptoms, requires mutant SOD1 (mSOD1) expression in other cells in addition to neurons (Jaarsma et al., 2008; Pramatarova et al., 2001). Expression of the mutant SOD1 protein in neurons and glia together is sufficient to cause cellular toxicity to the neurons, resulting in neuronal degeneration and eventual cell death (Beers et al., 2006; Boillee et al., 2006; Clement et al., 2003; Di Giorgio et al., 2007; Nagai et al., 2007; Yamanaka et al., 2008). This has been shown using both cell culture and *in vivo* experiments, and illustrates the importance of both astrocytes and microglia in ALS.

Astrocytes are the most populous cell type in the CNS. In addition to their obvious structural role, they are also intimately involved in the homeostasis of the extracellular environment, influencing neuronal excitability by modulating the levels of ions and neurotransmitters, and influencing neuronal health by releasing growth factors (Aschner et al., 2002; Bezzi and Volterra, 2001). In response to injury in the brain, astrocytes become activated. Whilst the terms "astrocytosis" or "gliosis" are often used non-specifically, a typical marker is up-regulation of the intermediate filament GFAP, and the accompanying morphological change, with hypertrophic nuclei and increased prominence of processes (Pekny and Nilsson, 2005). This measure has been noted in both *SOD1* mice and human ALS cases, both in the spinal cord and brain motor regions (Hall et al., 1998; Hirano, 1996; Kassa et al., 2009; Kushner et al., 1991; Nagata et al., 1998; Petrik et al., 2007). However, the onset of astrocyte activation is necessarily unclear in human cases, and varies between transgenic murine strains (earlier in mouse G85R and rat G93A, later in mouse G93A) (Alexianu et al., 2001; Bruijn et al., 1997; Hall et al., 1998; Nagai et al., 2001). Using a GFAP-luciferase reporter mouse, (Keller et al., 2009) found that the sequence of astrocyte activation even within one mouse model is complex, increasing and decreasing in waves at different times throughout the disease, within different levels of the neuraxis. Interestingly they found that the earliest change found was upregulation of GFAP in peripheral nerve Schwann cells, highlighting that pathology in the PNS may play an important role in ALS pathology. Other immunological changes at the level of the PNS have also been noted, including infiltration of humoral antibodies, complement and macrophages, strengthening the hypothesis that PNS pathology is important in ALS pathogenesis (Chiu et al., 2009).

Knowing when and where astrocytes become activated in the CNS is useful, but understanding the possible functional consequences, including activation of other cell types or direct neuronal death, is required. In one recent study, Diaz-Amarilla et al. (2011) created primary astrocyte cultures from symptomatic *SOD1* rat spinal cords in order to better understand the phenotype of "aberrant astrocytes." This identified a subset of astrocytes in symptomatic rats that showed high proliferation, expression of GFAP and other astrocyte markers, but in which there was an absence of GLT-1. This is consistent with findings of a decrease in GLT-1 expression around the time of spinal motor neuron loss in *SOD1* models (Bruijn et al., 2004; Howland et al., 2002). GLT-1 is involved in uptake of excess glutamate at astrocyte end-feet, so this decrease could contribute to the glutamate excitotoxicity noted in the disease (Cleveland and Rothstein, 2001; Rothstein et al., 1992). Indeed, glutamate levels have been found to be elevated in the CSF of sporadic ALS cases (Spreux-Varoquaux et al., 2002), and Riluzole – a TTX-sensitive Na-channel blocker, which prevents glutamate release by preventing calcium influx – is the only drug to offer any therapeutic effect in ALS patients (Bensimon et al., 1994; Meininger et al., 2000; Miller et al., 2003). Aberrant astrocytes also seem to produce soluble factors that are toxic to co-cultured motor neurons (Di Giorgio et al., 2008; Diaz-Amarilla et al., 2011; Marchetto et al., 2008; Nagai et al., 2007), but these are, as yet, mostly unidentified.

Activated astrocytes may also affect motor neurons via reduced release of trophic factors, which normally promote neuronal survival. Both *in vivo* and *in vitro*, a number of trophic factors, including BDNF, GDNF and VEGF have been shown to rescue motor neurons from various forms of insult (Ekestern, 2004; Van Den Bosch and Robberecht, 2008; Van Den Bosch et al., 2004). Reducing VEGF levels by deleting the hypoxia response element (HRE) of the VEGF gene in mice (Oosthuyse et al., 2001) caused motor neuron degeneration reminiscent of the *SOD1* model. Further evidence for the role of VEGF in ALS