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Introduction

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig’s disease, is a progressive neurodegenerative disorder characterized by loss of motor neurons in motor cortex, brainstem, and spinal cord that results in muscle weakness and atrophy, spasticity, and compromised speech, swallowing, and breathing. ALS is an invariably fatal disease, with patients typically dying within 3 to 5 years after symptom onset (1, 2). Most ALS cases are sporadic; i.e., patients do not have a family history of ALS and the cause of their disease is unknown. However, about 5%–10% of ALS cases have a family history of the disorder, typically with dominant inheritance. During the past decades, pathogenic mutations in a number of genes, including ORF 72 on chromosome 9 (C9orf72), superoxide dismutase 1 (SOD1), TAR DNA binding protein (TARDBP, also known as TDP-43), FUS RNA binding protein (FUS), heterogeneous nuclear ribonucleoprotein A1 (HNRNPA1), sequestosome 1 (SQSTM1), valosin-containing protein (VCP), optineurin (OPTN), TANK binding kinase 1 (TBK1), ubiquilin 2 (UBQLN2), and profilin 1 (PFN1), have been identified in ALS (3–18). These genes are involved in a variety of cellular pathways, including protein homeostasis, RNA metabolism, and cytoskeletal dynamics. Importantly, many apparently sporadic cases of ALS also have a genetic component: 1%–3% of sporadic ALS patients carry missense mutations in SODI (19), and roughly 5%–10% of the sporadic ALS are caused by intronic expansions in C9orf72 (20), with a larger number of cases carrying missense variants of unclear significance in one or more ALS genes (21). Several additional genetic variants have also been discovered that increase susceptibility to ALS and/or modify the clinical phenotype. For example, intermediate expansions to 27–33 CAG repeats in ataxin 2 (ATXN2) increase the risk of developing ALS (22), whereas reduced expression of the axonal guidance gene Eph receptor A4 (EPHA4) is associated with improved overall survival of people with ALS (23). These genetic insights provide the conceptual basis for most ideas regarding mechanisms underlying the initiation and progression of this devastating disease.

Using cellular and animal model systems based on genetic insights, a picture of the etiology and pathogenesis of ALS has started to develop, although the priming events that lead to disease initiation and mechanisms causing motor neuron degeneration still remain elusive. Numerous studies support the idea that neuronal cell death is multifactorial and includes mechanisms such as endoplasmic reticulum stress, mitochondrial dysfunction, oxidative stress, misfolded protein aggregation and impaired degradation, calcium overload, aberrant RNA/DNA regulation, and neuroinflammation (24, 25). Neuroinflammation is a pathological hallmark of ALS patients, and PET studies have shown that activated microglia are present in the brains of living ALS patients (26, 27). Early studies on ALS patient autopsies revealed that activated astrocytes, microglia, and infiltrating T cells are found at sites of motor neuron injury (28–30), suggesting that the immune system might have a role to play in the disease. More recently, identification of several ALS/frontotemporal dementia (FTD) genes, such as C9orf72, progranulin (PGRN), and TBK1, which are highly expressed in microglia and influence their function, has highlighted a potential direct role for these cells in ALS/FTD pathogenesis (18, 31–33). In this Review, we focus on the role of immune cells in the pathogenesis of ALS/FTD, and discuss epidemiological evidence for overlap between autoimmunity and ALS/FTD, suggesting shared genetic risk factors in these diseases.

Genetic connections between microglia and neurodegeneration

Microglia are the main cells in the CNS responsible for immune surveillance and are critical for normal development and homeostasis (34–36). Under steady-state conditions, “ramified” microglia continuously survey the local microenvironment for any foreign antigens and insults. The resident microglia are...
derived from early yolk sac precursors (37), and are distinct from circulating monocytes that can infiltrate into the brain in the setting of injury or neurodegeneration (38). Any insult or injury that disrupts brain homeostasis is sensed rapidly by microglia, resulting in a change in their morphology, and release of cytokines and chemokines to clear pathogens or debris in order to mitigate the damage caused by the injury (35). Upon insult, microglia upregulate expression levels of certain molecules, such as CD11b and lb1, and gain expression of molecules associated with antigen presentation, such as MHC-II, B7.1 (CD80), and B7.2 (CD86), which are absent in naive microglia (39). However, under chronic stress conditions such as progressive neurodegeneration and breakdown of the blood-brain barrier, there is an infiltration of other myeloid cells into the brain (39). Elegant studies have shown that these infiltrating cells present different gene expression profiles and are phenotypically different from resident microglia (39–41), but work together with the resident microglia to control the damage inflicted due to danger stimuli. Microglia with a morphologically activated phenotype are present in nearly all CNS diseases, including Alzheimer’s disease (AD), Parkinson’s disease, ALS/FTD, and prion diseases (42, 43). Given the nonspecific nature of microglial activation in neurodegeneration, it has long been debated whether it is a cause or a consequence of disease. However, the idea that microglia may play an active role in neurodegeneration has been strongly reinforced in recent years by the discovery of genetic variants in several immune genes that alter the risk of neurodegenerative diseases (44–46). Heterozygous mutations in triggering receptor expressed on myeloid cells 2 (TREM2) are known to increase the risk for various neurodegenerative disorders, including AD and possibly also ALS (47–49). TREM2 is exclusively expressed by microglia in the CNS (48, 50), and mutations in this gene likely confer loss of TREM2 protein function, thereby leading to altered microglial survival, phagocytosis, and inflammatory response (51, 52). Similarly, haplinsufficiency in another microglial-expressed gene, PGRN, is a common genetic cause of FTD, and also confers elevated risk of developing AD (53). PGRN acts as an inflammatory modulator and facilitates microglial phagocytosis of amyloid-β (Aβ) and apoptotic cells (54, 55). Lack of PGRN leads to dysregulation of microglial complement gene expression and loss of synaptic pruning and lysosome maturation (56). Several other genetic risk factors have also been identified that affect immune regulation and development of neurodegeneration. Leucine-rich repeat kinase 2 (LRRK2), which is mutated in Parkinson’s disease, is highly expressed in activated microglia and peripheral myeloid cells and is associated with several immune-related disorders (57). Apolipoprotein E allele ε4 (APOE*ε4), the strongest known genetic risk factor for AD, is highly expressed in microglia and has been shown to influence microglial activation in response to Aβ deposition (58). Microglia also express complement receptors and mediate synaptic pruning in the developing brain (59, 60). Classical complement protein C1q is induced in the brains of AD mice (61, 62) and mediates early synapse loss in AD mouse models (63). Interestingly, secretion of inflammatory proteins such as C1q and IL-1β by activated microglia leads to activation of astrocytes, which may be neurotoxic in neurodegenerative disorders (64). Polymorphisms in the genes encoding complement receptor 1 (CRI) (65) and clusterin (66) have also been hypothesized to influence receptor-mediated clearance of Aβ from the brain by microglial endocytosis. Subsequent studies in AD have identified five additional genes (CD33, the MS4A6–MS4A4 cluster, ATP-binding cassette-A7 [ABC7], CD2-associated protein [CD2AP], and EPH receptor A1 [EPHA1]), the products of which are all postulated to be involved in immune system regulation (reviewed in ref. 67). How these risk loci affect the functions of innate immune cells inside and outside of the CNS and how they increase susceptibility to neurodegeneration are not yet fully understood. Nevertheless, taken together, these studies provide strong genetic support for the concept that microglial dysfunction can directly influence the onset and progression of a variety of neurodegenerative disorders, including ALS/FTD.

Microglia in ALS: lessons from SOD1

Transgenic mice overexpressing mutant human SOD1 (mutSOD1) are the most commonly used model to study disease pathogenesis in ALS. These mice develop a progressive motor neuron disease that resembles the clinical and pathological features of human ALS. Studies have shown that overexpressing mutSOD1 only in motor neurons is not sufficient to fully manifest disease, indicating that other cell types are also required for neurodegeneration (68–71). An elegant study using chimeric mice further supported the importance of non-neuronal cells in motor neuron death: motor neurons lacking mutSOD1 developed features of ALS pathology when surrounded by mutSOD1-expressing glia, whereas motor neurons expressing mutSOD1 but surrounded by WT glia appeared healthy (72). While astrocytes, oligodendrocytes, and other cells contribute to disease pathogenesis, several studies support that microglia play an important role in non-cell-autonomous motor neuron degeneration.

Evidence from cultured microglia supports that mutSOD1 can have a cell-autonomous effect on their function, rendering them capable of promoting motor neuron death. LPS-activated WT microglia are cytotoxic to cocultured primary motor neurons owing to production of ROS (73). LPS-stimulated microglial cells carrying mutSOD1 are at baseline more activated than WT microglia, produce more superoxide, NO, and TNF-α, and are more injurious to primary cultured motor neurons (74, 75). Inhibition of NF-κB suppresses microglia-mediated neuroinflammatory toxicity when mutSOD1 microglial cells are cocultured with motor neurons (76). These results are further supported by data from transgenic mouse models overexpressing mutSOD1. In these animals, microglial activation in the spinal cord is evident prior to the onset of clinical weakness, increases as disease progresses, and is maintained through the late stages of disease (77, 78). Supporting that mutSOD1 can directly alter microglial function, removal of mutSOD1 transgene within Cd11b+ cells increased the lifespan of SOD1127R mice by approximately 100 days (79). Similarly, WT microglia extended survival in PU.1 knockout mice bred with SOD1109A mutants (80). Inhibition of IL-1β, which is produced by activated microglia, also attenuated inflammatory pathology and enhanced survival in mutSOD1 animals (81), supporting that microglial-produced cytokines directly influence disease progression.

A key question that arises from these studies is how microglia become activated in the first place. Microglia are the resident...
surveillance cells of the brain and are highly sensitive to changes in their environment. They can respond to endogenous danger signals derived from molecules released by damaged cells, stress-induced proteins, and abnormal protein accumulation in their surroundings to initiate an immune response. In the context of ALS, one possibility is that accumulation of misfolded proteins (such as mutant or oxidized SOD1) can actuate microglia through CD14/TLR2/TLR4 and scavenger receptor–dependent pathways (82–84). Expression of mutSOD1 in microglia is also known to produce excessive extracellular superoxide (85), which can damage the nearby neurons. It is important to note, however, that once activated, microglia can manifest either protective or damaging effects depending on stage and progression of the disease. For example, microglia from presymptomatic mutSOD1 mice exhibit an antiinflammatory phenotype and attenuated innate immune response compared with microglia from symptomatic mice, which are proinflammatory and exhibit toxic properties (86, 87). Similar findings were demonstrated in vitro (88), suggesting that an adaptive shift in functional microglial properties (86, 87). Similar findings were demonstrated in vitro (88), suggesting that an adaptive shift in functional microglial

**Figure 1. Schematic of the C9orf72 gene.** The location of the intronic hexanucleotide (GGGGCC) repeat expansion (black triangles) is shown between the noncoding exons 1a and 1b, upstream of the start codon. (A) Expansion of the hexanucleotide repeat from the normal range (about 2–30) to very large sizes (typically about 100–1,000) is the most common genetic cause of ALS/FTD. Bars represent coding (yellow) and noncoding (red) regions, and the positions of the start codon (ATG) and stop codon (TAA) are indicated. (B) Large GGGGCC expansions cause downregulation of C9orf72 gene expression, supporting that loss of normal C9orf72 function may contribute to disease. SALS, sporadic ALS; C9ALS, C9-associated ALS. RPKM, reads per kilobase per million. (C) Both sense and antisense transcripts containing C9orf72 expansions form RNA foci in cell nuclei, which may have a toxic effect on nuclear protein function. Repeat-containing transcripts can also serve as templates for RAN translation to generate simple dipeptide repeat proteins (RAN peptides) that accumulate in the brains of carriers and may contribute to disease through a variety of toxic mechanisms. C9orf72 expression data are based on data from Prudencio et al. (112). Sense and antisense foci and RAN dipeptide pictures are reproduced with permission from Neuron (147).

 phenotypes from neuroprotective to neurotoxic may accelerate disease progression in later stages.

**C9orf72 in ALS/FTD**

In 2011, the pathogenic expansion of a noncoding hexanucleotide repeat (GGGGCC) in C9orf72 was identified as the most common genetic cause of ALS and FTD, accounting for roughly 40% of familial ALS and 5%-10% of sporadic ALS cases (11, 14). Normal healthy individuals usually have 2–23 repeats, while affected individuals typically have repeat expansions in the hundreds or thousands. Given the major contribution of this repeat expansion mutation to both sporadic and familial ALS, there has been intense interest in deciphering the mechanisms by which GGGGCC repeat expansions cause neurodegeneration (89). Currently, three broad mechanisms are proposed to explain how these repeat expansions could cause disease (Figure 1). First, the presence of a large GGGGCC repeat expansion in the promoter region could cause a downregulation in C9orf72 expression, leading to a loss of C9orf72’s normal cellular function. Many reports have observed decreased C9orf72 transcript levels in tissues from mutation carriers (11, 90). Supporting the idea that haploinsufficiency could contribute to disease, complete loss of the Caenorhabditis elegans C9orf72 ortholog resulted in motor neuron degeneration and age-dependent deficits in motility (91). These mutants were also hypersensitive to environmental stress–induced neurodegeneration. Similarly, depletion of C9orf72 via antisense oligonucleotides in zebrafish resulted in axonal and motor defects, suggesting that loss of C9orf72 can directly impair motor neuron function (92). Second, RNA-mediated toxicity has been proposed due to the presence of sense (11) and antisense RNA foci (93) observed in cells from C9orf72 carrier brain and spinal cord samples. These RNA foci could sequester RNA-binding proteins, leading to splicing defects and alterations in RNA metabolism (94), but the exact identity of the proteins that bind to foci and are functionally altered remains elusive (95–97). Third, dipeptide repeat proteins that are produced by repeat-associated non-AUG (RAN) translation accumulate in the brain and spinal cord of C9orf72 mutation carriers (93, 98, 99) and have a variety of toxic properties that may drive neurodegeneration. Dipeptide repeat protein accumulations and/or RNA inclusions have been shown to impair nucleocytoplasmic transport in affected cells (100–102); however, ongoing studies are needed to determine the connection between nucleocytoplasmic transport defects and selective neuronal death in ALS/FTD. Potential gain-of-function mechanisms were recently reviewed elsewhere (103, 104).
C9orf72, myeloid cells, and immunity: an unexpected connection

Several studies of germline knockout of C9orf72 in mice were published last year that provide insights into how haploinsufficiency of C9orf72 could contribute to neurodegeneration (105–107). None of the mice in these studies showed neurodegeneration or motor system dysfunction consistent with ALS/FTD phenotypes, suggesting that C9orf72 function is not as critical in mammalian neurons as was observed in lower organisms. Instead, C9orf72−/− mice developed progressive splenomegaly and lymphadenopathy, altered splenic myeloid cell populations, increased levels of circulating proinflammatory cytokines, and in some colonies a fulminant autoimmune disorder (105, 106, 108). The effects seen upon C9orf72 deletion are perhaps not surpris-
C9orf72 leads to a systemic proinflammatory state likely driven by myeloid cells in the spleen and lymph nodes, which can result in autoimmune tissue injury depending on the environment; and (b) that haploinsufficiency is sufficient to alter myeloid cell function and systemic immunity in mice. The fact that loss of just one copy of C9orf72 can disrupt myeloid cell function has important implications for C9orf72-associated ALS/FTD, given that dominantly inherited C9orf72 expansions show only partial rather than complete loss of C9orf72 expression.

In addition to altered systemic immunity, C9orf72-deficient mice developed mild age-related neuroinflammation (93, 94). Similar to what was observed in C9orf72−/− macrophages, C9orf72−/− microglia showed lysosomal accumulation and a proinflammatory phenotype with increased expression of IL-6 and IL-1β (106). Transcriptional profiling of spinal cords from C9orf72−/− animals revealed age-related upregulation of inflammatory pathways, which were more similar to C9-associate FTD patient tissue than to sporadic FTD tissue (106, 112). This suggests that C9-associated FTD patients have enhanced microglial responses compared with sporadic FTD patients, which is consistent with a prior study showing enhanced microglial pathology in C9-associated FTD cases (113). Interestingly, an earlier experiment knocking down C9orf72 expression using intrathecal administration of antisense oligonucleotides in mice also showed upregulation of microglial activation genes, including Trem2, Clqα, and TYRO protein tyrosine kinases binding protein (TyrOPh), again without evidence of neurodegeneration (114). Taken together, these studies support the idea that decreased C9orf72 expression in microglia can directly alter their function and result in age-related neuroinflammation; however, in isolation, this altered microglial function is not sufficient to cause neurodegeneration, at least in rodents.

The findings of altered myeloid cell function and autoimmunity in C9orf72−/− mice raise an interesting question — do C9orf72 expansion carriers (or ALS/FTD patients in general) have altered immune responses or a tendency to autoimmunity? Interestingly, a variety of studies have investigated this question, and support the somewhat surprising notion that ALS/FTD and autoimmunity may have a shared etiology, as outlined below.

Inflammation and autoimmunity in ALS/FTD

The idea that autoimmunity is associated with ALS has been appreciated for decades. Seminal studies in the 1980s showed that ALS patients have a high frequency of monoclonal paraproteinemia (115) and a high incidence of autoimmune disorders (116). Numerous IgG antibodies and complement proteins are found in sera (117) from ALS patients, and antibody titer was positively associated with increased disease severity (118). Monoclonal IgGs were detectable in 60% of sporadic ALS patients (119), and autoantibodies against ganglioside GM1 and GD1a (120), sulfogluuronylparagloboside (121), neurofilament proteins (121), the TNF receptor family member FAS (CD95) (122), and voltage-gated Ca2+ channels (123, 124) have all been reported, indicating that ALS patients generate antibody responses to a variety of autoantigens.

More recent epidemiological and genetic studies continue to support the association between ALS and autoimmunity. A large epidemiological study found that there were significantly more cases than expected of ALS associated with a prior diagnosis of asthma, celiac disease, juvenile-onset diabetes, multiple sclerosis (MS), myasthenia gravis, systemic lupus erythematosus, and ulcerative colitis, supporting that autoimmune diseases and ALS might share common genetic or environmental risk factors (125). Other recent studies found an increased prevalence of autoimmune diseases in FTD with TDP-43 pathology (126), and specifically in C9orf72 patients with ALS/FTD (127). Interestingly, in a small cohort of patients with the rare combination of MS and ALS, approximately 80% carried a hexanucleotide repeat expansion in C9orf72 (128), suggesting these patients may have hyperactive immune responses to myelin antigens. Likewise, the recent identification of loss-of-function mutations in TBK1, a well-characterized regulator of innate immunity, as a cause of ALS/FTD (18, 129, 130) further reinforces the concept that immune dysregulation might be a characteristic feature of the disease.

Despite the strong case for a connection between ALS/FTD and autoimmunity, there is also convincing evidence against the idea that ALS is itself a classical autoimmune condition, given that administration of immunosuppressive drugs including corticosteroids, azathioprine, and cyclophosphamide (alone or in combination), as well as plasmapheresis or intravenous Igs (IVIgs), has failed to alter the progression of the disease (131-137). Several reasons have been posited to explain the failure of these drugs to slow disease progression in ALS patients. First, given that ALS is a heterogeneous disease, perhaps not all ALS patients have an inflammatory component. Second, since we do not know the dynamics and nature of the interaction between the immune system dysfunction and motor neuron death, we do not know whether administration of immunomodulatory therapies after disease onset may be too late, or even at a time when immune activation has a protective function. Finally, it is possible that instead of autoimmunity directly causing ALS/FTD, the two conditions arise from shared genetic or environmental risk factors and simply co-occur in the same individuals and families.

Owing to the dual effects of microglia in the CNS, being either protective or detrimental depending on expression of regulatory signals released in the local environment at different disease stages, targeting microglia therapeutically presents significant challenges. Additional longitudinal studies with comprehensive phenotyping of the immune system and a better understanding of how emerging genetic risk factors including C9orf72 and TBK1 influence immune cell function will be helpful to rationally design and test immunotherapies in ALS/FTD.

C9orf72 loss of function and inflammation: looking forward

Despite the explosion in research on the role of C9orf72 expansion in neurodegeneration, we have much to learn about the potential loss- and gain-of-function effects of C9orf72 on both neuroinflammation and neurodegeneration. Studies deciphering the molecular functions of C9orf72 are helping define the effects of loss in different cell types, including neurons and microglia. Bioinformatic analysis has shown that C9orf72 contains a differentially expressed in normal and neoplastic cells (DENN) domain (138) and is hypothesized to play a role in intracellular trafficking and autophagy and lysosomal functions. Moreover, upon C9orf72 depletion, endocytosis was found to be impaired and autophago-
some formation to be dysregulated (139), suggesting that C9orf72 plays a critical role in autophagy and endosomal-lysosomal pathways. C9orf72 was also shown to interact with the TBK1 targets Smith-Magenis syndrome chromosome region, candidate 8 (SMCR8), and WD repeat domain 41 (WDR41) (140–142), suggesting that C9orf72 and TBK1 may act within the same pathway. As discussed earlier, mutations in a number of genes known to be involved in autophagy and lysosomal pathways are linked to ALS/FTD, including TBK1, OPTN, VCP, PGRN, UBQLN2, charged multisvesicular body protein 2B (CHMP2B), and SQSTM1 (8–10, 129, 143, 144). The ubiquitous presence of ubiquitin- and p62-positive inclusions in ALS/FTD cases further supports the idea that an impaired autophagosome-lysosome system plays a role in disease. Given that these genes are expressed in both microglia and neurons, it is possible that mutations in this cluster of ALS/FTD genes have a common ability to promote aberrant protein aggregation in neurons (toxic gain of function) and abnormal inflammatory responses in microglia (loss of C9orf72 function), which uniquely combine to result in neurodegeneration (Figure 2). Likewise, alterations in this gene cluster may be partly responsible for the shared tendency toward both autoimmunity and neurodegeneration in ALS/FTD. Continued experimental work is needed to address some key questions moving forward. First, which cell types exhibit loss- and gain-of-function manifestations of C9orf72 expansion in human patient tissues, and do some cells manifest both gain and loss of function? Studies on brain tissue showing reduced C9orf72 expression have primarily looked at the RNA level, and were not able to determine which cell types contribute to the overall decrease in expression. Moving forward it will be helpful to incorporate better antibodies to examine protein levels, and to examine expression and epigenetic modification at the cellular rather than the tissue level. Second, can altered immune responses be measured in cells from C9orf72 expansion carriers, and are loss-of-function mutations in C9orf72 enriched in any autoimmune conditions? Such data would help us to understand whether the decrease in C9orf72 expression (around 50%) seen in patients has similar immunological consequences to those observed in the rodent models. Third, can combined loss- and gain-of-function alterations in C9orf72 be demonstrated in cell- or animal-based experimental models?

Answering these questions will have immediate implications for therapeutics, given that one of the primary strategies being developed to treat C9orf72-associated ALS/FTD is knockdown of the toxic C9orf72 species using antisense oligonucleotide technology (114, 145, 146). Therefore, a deeper understanding of the effect of loss of C9orf72 function on immune cells will be important both to interpret the results of knockdown studies in humans and to properly monitor patients for potential adverse effects.

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