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REVIEW

Infectious agents and amyotrophic lateral sclerosis: another piece of the puzzle of motor neuron degeneration

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Abstract

Amyotrophic lateral sclerosis (ALS) is the most common neurodegenerative disease affecting motor neurons (MN). This fatal disease is characterized by progressive muscle wasting and lacks an effective treatment. ALS pathogenesis has not been elucidated yet. In a small proportion of ALS patients, the disease has a familial origin, related to mutations in specific genes, which directly result in MN degeneration. By contrast, the vast majority of cases are though to be sporadic, in which genes and environment interact leading to disease in genetically predisposed individuals. Lately, the role of the environment has gained relevance in this field and an extensive list of environmental conditions have been postulated to be involved in ALS. Among them, infectious agents, particularly viruses, have been suggested to play an important role in the pathogenesis of the disease. These agents could act by interacting with some crucial pathways in MN degeneration, such as gene processing, oxidative stress or neuroinflammation. In this article, we will review the main studies about the involvement of microorganisms in ALS, subsequently discussing their potential pathogenic effect and integrating them as another piece in the puzzle of ALS pathogenesis.

Keywords ALS · Amyotrophic lateral sclerosis · Bacteria · Fungi · Infection · Microorganisms · Parasites · Viruses

Introduction

Amyotrophic lateral sclerosis (ALS) is the most common neurodegenerative disease affecting motor neurons (MNs) [1, 2]. It is characterized by progressive upper and lower MN degeneration that typically lead to death within 3 years after symptom onset [3].

The pathogenesis of ALS has not been fully elucidated yet. However, our knowledge about disease mechanisms has significantly improved in the last few years [1, 4]. In this regard, the impairment of various cellular functions and signalling pathways has been related to MN degeneration [1, 5]. Among them, abnormalities in gene processing, proteostasis, and axonal transport, as well as the involvement of glial cells surrounding MNs seem to be relevant mechanisms [6]. ALS cases can be divided into familiar (fALS) and sporadic (sALS) fALS represent a small percentage of cases. They are secondary to mutations of specific genes (Cr9orf72, SOD1, FUS, TDP43, etc) which directly induce MN degeneration and disease [1]. By contrast, most cases are thought to be sporadic (sALS). In sALS, genes and environment interact each other leading to disease onset and progression in genetically predisposed individuals [7]. In line with this concept, the role of environmental factors in the pathogenesis of ALS is receiving increasing attention [8]. There is a wide list of environmental conditions that have been potentially associated to ALS [7]. Interestingly, apart from the classic risk factors for ALS, as the exposure to heavy metals or the extenuating physical activity, new categories have been recently considered [1]. In this line, infectious agents, including viruses, bacteria and fungi, are increasingly considered to have a role in the pathogenesis of the disease. In this article, we will review the most relevant

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studies discussing the role of infections in the disease, thus representing another piece in the ALS pathogenesis puzzle.

The role of viruses

Viruses are among the most frequent microorganisms in human environments. Differently from other pathogens, such as bacteria, viruses require a host cell for their replication. These microorganisms characteristically show a marked variability, which sometimes hampers the immune host response [9]. Some viral infections have an acute course (for example, those due to influenza or syncytial respiratory virus) while others develop chronically, such as those induced by human immunodeficiency virus or hepatitis c virus. In the last decades, viral infections, particularly chronic ones, have been associated to different conditions, including immune, cardiovascular and neurological diseases [9, 10].

Regarding ALS, viruses are probably the microorganisms whose implications in ALS pathogenesis have been most extensively studied. Remarkably, persistent viral infections have been pointed out as a predisposing factor of ALS. The most representative studies are summarized in Table 1. Next, we will discuss some important ones.

Retroviruses

Retroviruses have been associated with ALS-like syndromes. In this line, upper motor neuron manifestations appear in a small percentage of patients infected with HIV-1 and HTLV-1 viruses [11–13]. However, there are major differences between the so-called ALS-like syndromes and the classical form of ALS [11, 14]: patients are younger at the time of onset, cerebrospinal fluid (CSF) pleocytosis may be present [15], there is no inexorable progression, and more importantly, the syndrome ameliorated after the institution of anti-viral therapy [13, 16].

HIV

During the last 30 years, more than 20 cases of ALS/ALS-like disease have been reported in HIV seropositive individuals [17]. Reverse transcriptase (RT), a critical retroviral enzyme, has been detected in ALS patients' sera than in controls [13, 16]. However, RT activity is hardly detected in CSF of ALS patients. Some authors have speculated that RT CSF levels might be influenced by systemic viral replication [16].

The mechanism by which HIV infection leads to ALS-like syndromes remains unclear. HIV infects macrophages, microglia and astrocytes, but does not infect neurons, therefore the neuronal damage is likely indirect, and may occur secondary to the effect of neurotoxic viral proteins, cytokines, chemokines, and oxidative stress, produced as a consequence of the viral infection [6, 18]. According to Louboutin and Strayer [19], HIV-infected monocytes and T cells need the gp120 to get inside the central nervous system (CNS); a glycoprotein that can directly induce apoptosis in neurons and increase oxidative stress [19, 20]. On the other hand, nuclear TDP-43 expression, the hallmark protein in ALS pathogenesis, was enhanced about sixfold in HIV patients' cortical neurons, along with enhanced TDP-43 phosphorylation [21].

HTLV-1

Westarp et al. reported that 50% of 50 patients with sporadic ALS had d antibodies reacting against HTLV-1/2 antigens [22]. However, other studies failed to find proviral DNA of HTLV-1 and2 in the CNS of patients with ALS [23, 24]. An autopsy study of one patient with ALS-like syndrome who was infected with HTLV-1 showed discrete lymphocytic infiltrates throughout the CNS, along with moderate anterior horn cell loss and both axonal and myelin losses in the lateral corticospinal tract at all spinal levels [25].

The real mechanisms by which HTLV-1 causes neurological diseases remains a mystery. There are three main hypotheses [12]: (1) direct toxicity (the expression of viral HTLV-1 antigens by infected glial cells, recognized by CD8, provokes the release of a plethora of cytokines, detrimental to motor neurons); (2) autoimmunity (inappropriate glial cell antigen recognition activates the immune cascade); and (3) "bystander damage" (cytotoxic T CD8 cells recognize interferon-gamma-secreting HTLV-1-infected CD4 T cells, and as a consequence, microglia secretes myelinotoxic cytokines). Recently, some investigators suggested that the production of interferon gamma by HTLV-1-infected cells in the CNS would provoke astrocytes to secrete the chemokine CXCL10, able to engage more infected cells via the chemokine receptor CXCR3, resulting in chronic inflammation [12].

Human endogenous retrovirus (HERV)

Human endogenous retrovirus (HERV) sequences, which represent up to 10% of human genomic DNA [19], might be pathogenic in ALS [26]. Besides, expression of distinctive transcripts encoded by the HERV-K genome is increased in brain tissue of patients with sporadic ALS [26–29], preferentially in areas adjacent to the motor cortex. In those cases, immunostaining revealed the expression of HERV-K in neurons but not in non-neuronal cells, in either postmortem brain tissue or in individuals with Alzheimer's disease [27, 29].

Table 1 Main studies supporting the role of viruses in ALS

Virus	Author	Study design	Main results
HIV	McCormick	Quantitative assay in serum and CSF from ALS patients and controls	Reverse transcriptase activity has been found to be positive more frequently in ALS patients' sera, but not in CSF
	Regulier	Studies in cortical cell cultures, in rat hippocampal slices and by intracerebral injections in vivo	Gp120 can induce apoptosis in neurons
	Douville	Autopsy tissue studies in HIV patients	Increased nuclear TDP-43 expression in HIV patients' cortical neurons
HTLV-1/2	Westarp	Measurement of circulating IgG immune complexes and altered IgG sero-reactivities against HIV-2 and HTLV in sALS patients	50% of sALS patients showed antibody reactivities against HTLV-1/2 antigens
	Silva	Autopsy study in an ALS-like syndrome infected with HTLV-1 virus	Lymphocytic infiltrates throughout the CNS, moderate anterior horn cell loss and both axonal and myelin losses in the lateral corticospinal tract at all spinal levels
HERV	McCormick	Quantitative assay in serum and CSF from ALS patients and controls	Elevated RT activity non-correspondent to exogenous already known retroviruses has been observed in both blood and cerebrospinal fluids in ALS patients
	Li	Autopsy brain tissue studies of patients with sALS compared to controls	Expression of distinctive transcripts encoded by the HERV-K genome is increased in neurons but not in non-neuronal cells
		In vitro studies using human-cultured neurons from ALS patients	Transfection of either the whole HERV-K genome or just its envelope protein triggered neurite beading and retraction, and eventually neuronal death
		Utero electroporation of *env* gene into embryonic mouse brain	HERVK envelope protein is neurotoxic in vivo
		Cotransfection of HERVK and TDP-43 into HeLa cells, followed by knockdown of endogenous TDP-43 with siRNA	Increased replication of HERV-K after cotransfection. Knockdown decreased HERV-K expression
	Douville	Autopsy tissue studies in HIV patients	Elevation of HERV-K RT expression in brain tissue with p24 reactivity
	Contreras-Galindo R	Real-time PCR quantitation of HERV-K RNA load in plasma samples	Enhanced HERV-K levels in patients who failed to respond to HAART therapy or were treated with sub-optimal therapeutic doses
		Longitudinal analysis of blood mononuclear cells in vitro. Evaluation of HERV-K in HIV-1-infected and control cells	HERV-K expression precedes spikes of HIV replication in select individuals
Coxsackievirus B3 (CV B3)	Fung	Evaluation of TDP43 in an in vitro assay in HeLa cell upon incubation with CVB3	CVB3 infection leads to cytoplasmic TDP-43 translocation
Echovirus-7	Cermerlli	Measurement of antibodies to echovirus-7 in sera of patients diagnosed with sporadic ALS	Positive results for echovirus-7 of 40% among referents and 55% in ALS patients

In vitro studies showed that transfection of either the whole HERV-K genome or just its envelope protein into cultured human neurons from ALS patients triggered neurite beading and retraction, and eventually neuronal death. The HERV-K envelope protein was also documented to be neurotoxic in vivo [26, 27, 29]. Moreover, the interaction of TDP-43 with the HERV-K long terminal repeat augments the expression of HERV-K genes, meaning that TDP-43 protein regulates HERV-K viral protein accumulation [21, 26–28]. Local neuroinflammation is likely a key driver of HERV-K expression in the brain, and the analysis of the HERV-K promoter suggests that inflammatory mediators may also modulate HERV-K's transcription [21, 27, 29].

Interestingly, there is a link in the literature between HIV and HERV-K. Independent groups have discovered that HERV-K proteins accumulate in cortical neurons of patients with HIV infection [21, 30]. The degree of HIV replication can be evaluated by the presence or absence of HIV p24 positive cells by immunochemistry. HERV-K RT expression was significantly elevated in brain tissue with p24 reactivity [21]. Moreover, enhanced HERV-K levels were observed in patients who failed to respond to HAART therapy or were treated with sub-optimal therapeutic doses. Furthermore, longitudinal analyses of peripheral blood mononuclear cells (PBMC) from HIV-infected patients show that increased HERV-K expression may precede spikes of HIV replication [21, 31].

Additionally, there might be a bidirectional interaction between viruses and damaged neuronal proteins. In fact, the enhanced neuronal expression of HERV-K may be activated by Tau protein released from HIV-infected cells [14, 21, 32]. Finally, some authors have hypothesized about the role of retroviruses in cell-to-cell transport of pathogenic TDP43, since exosomes can carry retroviral cargo [21, 33]. As suggested by Douville et al. [13], monitoring exosome composition could lead to the discovery of clinical biomarkers required for future studies on ERV-associated diseases [21].

Other viral agents

Enterovirus

Given the known tropism of poliovirus for MN, it seems reasonable to speculate that enteroviruses might play a role in ALS pathogenesis. Indeed, persistent infection by enterovirus has been reported to cause sporadic ALS. According to Ravits [34], six RT-PCR studies in ALS patients searching enteroviruses have been reported, and half of them showed a positive results.

Fung et al. proposed a model, based on the observation of TDP-43 translocation from the nucleus during infection, by which coxsackievirus B3 (CVB3) leads to cytoplasmic TDP-43 translocation, which is often accompanied by reduced solubility and increased formation of protein aggregates which result in neuron toxicity [35].

The seroprevalence of another enterovirus, Echovirus-7, was studied by Cermelli et al. by a neutralization test. They found anti-viral antibodies in 40% controls and 55% ALS patients [36].

Herpes viruses

Finnen et al. found that cells infected with herpes simplex virus 2 (HSV-2) failed to accumulate stress granules (SGs) (a physiological cell reaction) in response to arsenite, a chemical compound that induces oxidative stress [37]. In another study, it was demonstrated that the disruption of arsenite-induced SG formation by HSV-2 is mediated by a virion component [38], which is required for this disruption.

In conclusion, a definite etiologic link between viruses and ALS has not been established yet. However, many published data suggest an underlying connection (Fig. 1). Yet, we are just starting to gather the pieces of the complex puzzle that sporadic ALS is. Unveiling viral cellular pathways involved in ALS could further lead to new insights in ALS pathogenesis, and hopefully, open the door to upcoming therapies.

The role of bacteria

Although viruses may be the microorganisms postulated to play the main role in ALS pathogenesis, several studies also suggest a potential role of bacteria in ALS. Among bacterial infections, cyanobacteria and Lyme disease seem to be the most relevant ones. The role of the microbioma is another emerging field.

Cyanobacteria

Cyanobacteria and the beta-*N*-methylamino-L-alanine (BMAA) toxin, which has been identified in more than 20 cyanobacterial genera, constitute one the first environmental factors known to have a role in ALS. They are probably the most extensively studied. Evidence came from studies in Guam in 1945, where a disproportionate incidence of an ALS complex syndrome, which also included dementia and parkinsonian features, was reported among the Chamorro's population [39]. Two decades later, the neurotoxin b-*N*-methylamino-L-alanine (BMAA) was discovered in the indigenous cycad (*Cycas micronesica*), the seeds of which were used by Chamorros to make flour [40, 41]. Not long after, the causality principle was more consistently confirmed when the BMAA was detected in brains of Guamanian patients with ALS, but not in Guamanian control brains [42, 43]. Further studies demonstrated that BMAA in cycads

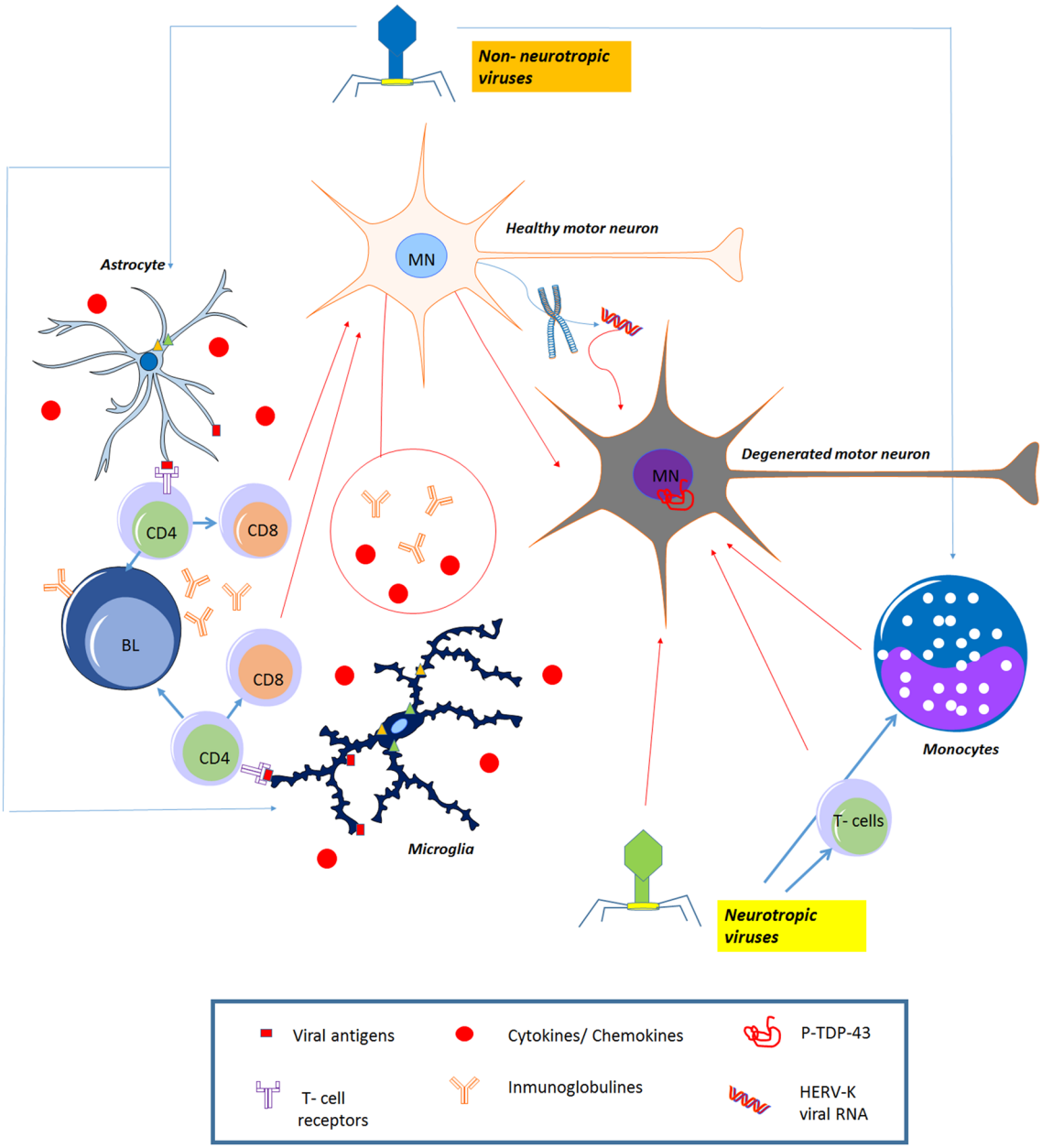

Fig. 1 An integrated perspective of viruses and motor neuron degeneration. Viruses may yield motor neuron (MN) degeneration by acting both directly (apoptosis' induction) and indirectly (by means of glial cells' activation and neuroinflammation). Non-neurotropic viruses, such as HIV or HTLV viruses, infect astrocytes and microglia, but peripheral monocytes and T cells as well. Glial cell infection entails two main consequences: cytokine/chemokine release and adaptive immune response cells activation in situ. As a result of the latter, immunoglobulin production and CD8 T cell activation occur. Both cytokine/chemokine generation and CD8 activation lead to MN degeneration, even reproducing some of the molecular hallmarks of ALS pathogenesis, pre-eminently TDP-43 phosphorylation and subsequent protein aggregation. One of the underlying molecular processes behind MN death secondary to neuroinflammation is the activation of the "dormant" HERV-K viral sequences. On the other hand, when infecting peripheral immune adaptive and innate cells, non-neurotropic viruses can yield motor neuron degeneration by several mechanisms, but mainly through direct apoptosis and increased levels of oxidative stress. Regarding neurotropic viruses, including enteroviruses or herpes viruses, evidence on how they may be responsible for motor neuron toxicity is fewer; however, it has been observed that they might play some kind of role in disrupting stress granule formation in response to oxidative stress, as well as in TDP-43 phosphorylation and aggregation

comes from symbiotic cyanobacteria resident in specialized coralloid roots of this plant, and that it gets concentrated during the process of flour production [42].

The role of BMAA in motor neuron disease has been further supported by other clusters reported in France and USA [44–47]. The precise mechanism explaining BMAA toxicity has not been completely clarified. It has been proved to induce neurotoxicity in both mice and non-human primates [48], resulting in MN death at concentrations of 10–30 Mm [49]. Interestingly, the BMAA toxin has also been involved in other neurodegenerative diseases such as Parkinson's disease or Alzheimer's disease [50]. However, there is not a clear concordance among studies, since some authors have reported inconsistent data in "in vivo" studies [51]. Regarding the pathogenic mechanisms, it has been postulated that BMAA behaves as an endogenous neurotoxic reservoir that would be slowly released within the brain and spinal cord inducing its neurotoxic effects over the years [42]. Complementary experimental studies have shown that the neurotoxic effects of BMAA might be related to the depletion of glutathione, glutamatergic toxicity, synergism with other neurotoxins, its capacity to induce protein misfolding, and accumulation of intracellular aggregates [52]. The interindividual BMAA susceptibility has been assessed by several investigators, but they have not been able to identify the genetic variants responsible for susceptibility yet [53].

Borrelia burgdorferi

Lyme disease, caused by the spirochete *Borrelia burgdorferi*, may present with a wide constellation of abnormalities from both the central nervous system (CNS) and the peripheral nervous system (PNS). Regarding the latter, polyradiculoneuropathy is one of the most common manifestations of the disease, which exceptionally might mimic ALS [54]. In addition, some studies have been carried out to explore the relationship between Borrelia infection and the risk of developing ALS [55]. The results do not support a causal link between infection and ALS. In this line, Visser et al. reported no association between *B. burgdorferi* antibodies and ALS. In fact, they found similar proportion of subjects with positive serology among ALS patients and the general population. In addition, they reported that, among ALS patients, the seropositivity condition was not associated with differences in survival [56]. Likewise, Muddasir et al. did not find differences in seropositivity rates. Furthermore, treatment with ceftriaxone was not associated with better prognosis of the disease [57].

Microbiota

The term microbiota refers to an "ecological community" of commensal symbiotic and pathogenic microorganisms. It includes bacteria, archaea, protists, fungi and viruses. Microbiota has been widely studied in recent years, as it has been postulated to have a pathogenic role not only in intestinal diseases but also in other immunological, inflammatory and degenerative disorders. These studies emphasize the role of microbiota in the immunologic, hormonal and metabolic homeostasis of the host [58, 59].

Regarding the nervous system, it has been reported that microbiota regulates the blood–brain barrier permeability and several cellular pathways, including neurone survival, neurogenesis and cell growth and differentiation [59]. On this basis, the relationship between microbiota and neurodegenerative diseases is receiving increasing attention [60]. To date, the majority of studies have included patients with Parkinson's disease and they reported differences in microbiota composition between patients and healthy controls, thus suggesting a role of microbiota in the neurodegeneration process.

A fewer number of studies have addressed the potential role of microbiota in ALS, but there is incomplete agreement among them. Regarding experimental models, some authors reported that the superoxide dismutase (SOD) enzyme activity, one of the most relevant proteins involved in ALS pathogenesis, could be modulated by intestinal microflora [61, 62]. Shaoping et al. examined the gut of SOD1G93A transgenic mice, which express a mutated form of SOD, and reported increased permeability and a reduction in the expression of some proteins, such as E-cadherin [63]. These changes were associated with a significant reduction in several microorganism including *E. coli, Fermicus* and *Butyrivibrio fibrisolvens* [63]. Interestingly, on this basis Zhang et al. reported that the administration of butyrate (a short chain fatty acid derived from bacteria) restored ALS-related dysbiosis and slowed disease progression in the SOD1 murine model [64].

The studies with ALS patients are also scarce. Fang et al. evaluated six ALS patients and described differences in indices and taxa species [65]. More recently, Brenner et al. studied 25 patients with ALS (23 sALS and 2 fALS) without bulbar symptoms or marked functional disability [66]. They found significant differences between ALS and healthy controls in both the overall number of microbial species and in the abundance of uncultured Ruminococcaceae [66].

In view of those preliminary and inconsistent data, future studies including larger cohorts of patients with different forms of the disease are needed to characterize ALS microbiota and to elucidate whether it plays an important role in the pathogenesis of the disease.

Other bacterial agents

Apart from those previously discussed the role of other bacteria species in ALS has been assessed without consistent

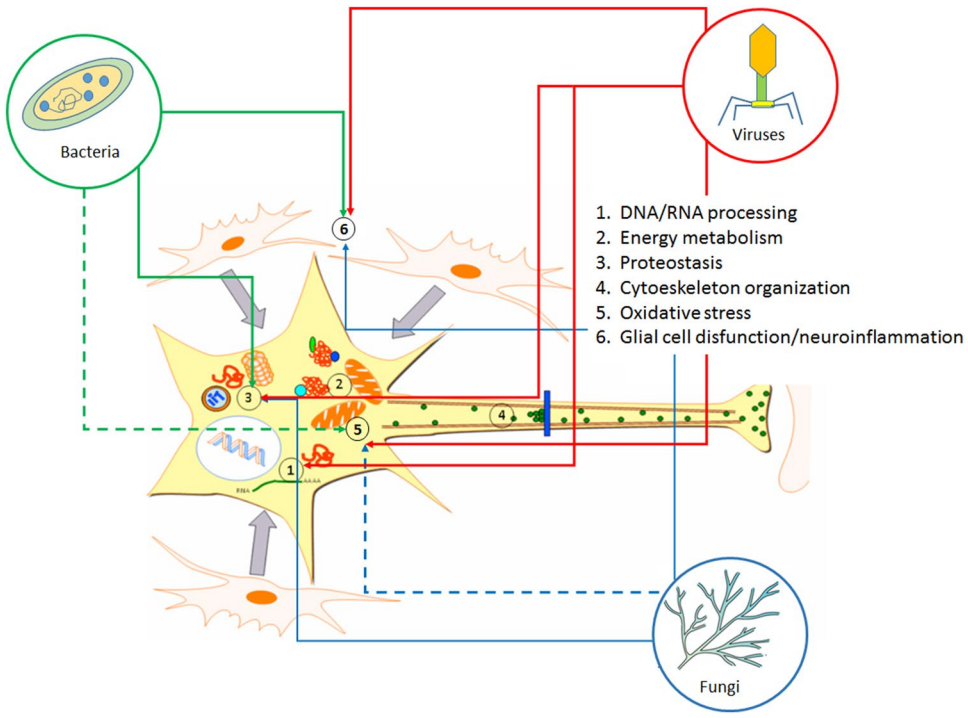

Fig. 2 Integrating microorganisms in ALS pathogenesis. Gene processing, energy metabolism, proteostasis, cytoskeleton organization, oxidative stress, glial cell dysfunction and neuroinflammation are regarded as important cellular pathways associated to motor neuron degeneration. Interestingly, some of them seem to be modified by several microorganisms. The figure shows current evidences supporting the involvement of microorganisms on these pathways. Dotted lines reflect those situations with weaker evidences

results. For example, increased rates of mycoplasma infection have been reported in Gulf War veterans and civilians diagnosed with ALS suggesting a pathogenic role in the disease [67]. However, other authors have not replicated that results [68].

The role of fungi

Fungi have a worldwide distribution and are able to grow and develop in a wide range of habitats and conditions. More than 100,000 species of fungi have been described, but the global biodiversity of the fungus kingdom has not fully elucidated yet. Some estimations suggest that there might be up to 3.8 million species. Currently, fungal species are classified according to morphology, genetics, biochemical and physiological features [69]. As it occurs with other microorganisms, a large proportion of them are commensal, exerting beneficial influences on neighbor organisms, while others can be highly deleterious.

Apart from their obvious role in infectious disorders, fungi have been associated to several non-infectious diseases [70, 71]. Regarding neurological disorders, some studies support their role in both neurodegenerative and inflammatory diseases, such as Alzheimer's disease or multiple sclerosis [72]. In this line, several fungi species have been identified in brains from Alzheimer's patients and in blood and cerebrospinal fluids from multiple sclerosis patients, thus suggesting that chronic fungal infection might act as a trigger or a predisposing condition of these diseases [72–74].

Carrasco et al. first suggested the implication of fungal infections in ALS after identifying fungal proteins and DNA traces in the cerebrospinal fluid and brain tissue of patients with ALS [75]. Not long after, the same investigators reported the presence of fungal structures, such as yeast and hyphae, in the motor cortex, the medulla and the spinal cord of 11 ALS patients. Different fungi species were identified by qPCR and next-generation sequencing (NGS). Among them, the most prevalent species were *Malassezia, Candida, Cryptococcus, Penicillium, Cladosporium* and *Davidiella* [76]. However, there was no concordance in the identified fungal species across patients, showing a high interindividual variability. In addition, the fungal species also varied according to the CNS localization studied (Motor cortex: *Candida, Malassezia* and *Fusarium*; Medulla: *Candida, fungus clone S24T, Basidiomycota; Spinal Cord: Candida, Fusarium, Malassezia*). Based on this diversity, authors speculated that these variations in fungal species might determine different forms of disease and prognosis [76].

Several mechanisms could link fungi with ALS: (1) protein metabolism, one of the most important pathway in ALS might be altered in those fungal-infected neurons; (2) neuroinflammation involving T lymphocytes infiltrates, which is often seen in ALS patients, could reflect some degree of fungal infection [77], (3) high levels of chitinase (an enzyme linked to fungal infection) have been reported

in ALS patients [75]; and (4) SOD1 protein, a commonly altered protein in ALS patients plays an important role in the immune defense against fungi [78]. However, in our view, there is not currently sufficient evidence for a role of fungi in ALS and further studies with larger sample size are needed before reaching definite conclusions.

The role of parasites

Regarding parasites, there is no clear evidence of a causal association between them and ALS. Anecdotally, Harvey et al. reported a patient with evidence of Borrelia and Babesia coinfection, who developed a progressive motor neuron disease consistent with ALS. Clinical manifestations improved after treatment with ceftriaxone and anti-Babesia therapy [79]. To the best of our knowledge, no other studies linked babesia infection with ALS.

Integrating microorganisms in the puzzle of ALS

Despite the huge efforts of the last decades, we still do not completely understand why MNs degenerate [1, 7, 80]. The most widely accepted theory, based on the gene–environment interaction concept, proposes that MN degeneration occurs in individuals in which aging-related cellular damage, environmental conditions and genetic risk factors add up reaching a certain threshold [7]. On this basis, the exposure to several environmental factors may induce ALS development.

Infections, and in particular some specific microorganisms including some viruses, bacteria and fungi, are suggested as new players in the pathogenesis of the disease (Fig. 2). In this scenario, microorganisms might be considered as environmental factors, which, in the presence of other external or genetic risk factors, could lead to ALS development. Interestingly, it has been reported that these agents might interfere in some crucial cellular MN pathways [6] including gene processing, proteostasis or in the glial cell-MN interaction.

This should not be confused with the fact that some infections, particularly Lyme disease, may show clinical manifestations of MN disease, thus occasionally mimicking ALS [54].

Although the role of microorganisms in the pathogenesis of the disease seems moderately supported, particularly in the case of viruses, there is currently no indication to treat patients with antimicrobial drugs. In the future, studies involving larger number of patients will help to precisely elucidate the importance of microorganisms, such as viruses or fungi, in ALS, addressing some crucial unresolved questions, such as if they act as a trigger or as perpetuating agents once the disease has already been established. The investigation of this new environmental category will doubtlessly provide us more knowledge about the disease, not only from a specific infectious point of view, but also from a more holistic perspective.

Compliance with ethical standards

Conflicts of interest All authors declare that they have no conflict of interests.

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