New Kid on the Block: Does Histamine Get Along with Inflammation in Amyotrophic Lateral Sclerosis?

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Abstract: Results from amyotrophic lateral sclerosis (ALS) patients and pre-clinical studies strongly suggest that systemic and CNS-intrinsic immune activation plays a central role in ALS pathogenesis. Microglial cells are emerging in this context as master regulators with a bi-functional role in the progression of the pathological response. They foster a pro-inflammatory setting through the production of cytotoxic cytokines and chemokines (M1 phenotype), after an aborted effort to sustain an anti-inflammatory environment for motor neurons through the release of beneficial cytokines and growth factors (M2 phenotype). In this review, we gather information meant to propose that histamine and ATP, which are released from mast cells, microglia and damaged neurons at sites of injury where they function as transmitters, have to be considered as new players in the ALS neuroinflammatory arena. After all, abnormal histamine and ATP signalling in the brain are already documented in neurodegenerative/neuroinflammatory conditions such as multiple sclerosis, Alzheimer and Parkinson's disease and, at present, histamine- as well as ATP-related compounds are in clinical trial for these same pathologies. Concerning ALS, while emerging data are now available about purinergic mechanisms, the involvement of histamine is basically unexplored. The circumstantial evidence that we present here thus constitutes a solid background for formulating novel hypotheses, stimulating a scientific debate and, most of all, inspiring future research. We deem that a new potential role of histamine in the setting of ALS neuroinflammation might find a fertile ground where to thrive. ALS is still a disease without a cure: why not to play with a new kid on the block?

Keywords: Amyotrophic lateral sclerosis, histamine, microglia, P2X7 receptor.

AMYOTROPHIC LATERAL SCLEROSIS

Overview

Amyotrophic lateral sclerosis (ALS) is one among the most destructive forms of adult-onset motor neuron degeneration, characterized by clinical marks such as muscle weakness, atrophy, spasticity, dysarthria, dysphagia and paralysis because of total denervation. When disease progresses, ALS patients fail in initiating and controlling most of their voluntary movements, but their sensory/cognitive functions are inexorably spared. Death usually occurs by respiratory failure after about one to five years from onset [1]. At the cellular level, ALS causes rapidly progressive injury and death of upper and lower motor neurons and their connections, with impairment of target muscle cells. The neurodegenerative process is accompanied by a sustained inflammation in the brain and spinal cord, and the neuron-immune interaction involving both CNS-resident microglia and blood-derived immune cells, is highly dynamic and complex over the course of the disease.

It is accepted that ALS is a multi-factorial pathology, attributable to and influenced by several as yet unidentified susceptibility genes and environmental factors. About 90% of ALS cases are sporadic (sALS), but the pathogenesis of the disease remains still unknown. In about 20% of familial ALS cases (fALS), over 100 different mutations have been found on chromosome 21q22.1, in the gene (*ALS1*) encoding the cytosolic copper-zinc superoxide dismutase (SOD1) enzyme. Several ALS genes and additional chromosomal loci have been recognized [2], and approximately 70% of familial cases are triggered by mutations of only four genes, *C9ORF72*, *SOD1*, *TARDBP* and *FUS*. Other major genes causing ALS are: *ALSIN* on chromosome 2q33 (ALS2); *SETX* on chromosome 9q34 (ALS4); *VAPB* on chromosome 20q13.3 (ALS8); *ANG* on chromosome 14q11 (ALS9) and *VCP* on chromosome 9p13.3 [3-6]. Recently, a massive hexanucleotide-repeat expansion (GGGGCC)n in the first intron of the *C9ORF72* gene has been reported as a major causative trait for ALS [7, 8]. However, the final proof of the pathogenic impact of these various mutations in ALS is still vaguely defined, and alterations of more than one ALS-related gene in the same patient are often described in both familial and sporadic forms of the disease, further providing evidence of the multigenic and multifactorial nature of ALS [9].

Molecular Mechanisms

At the molecular level, the mechanisms causing and/or propagating ALS are diverse, including glutamate-mediated excitotoxicity, mitochondrial dysfunction, oxidative and endoplasmic reticulum stress, aberrant axonal transport, protein misfolding and aggregation, dysfunction of the

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ubiquitin proteasome system, dysregulated RNA metabolism, alteration of cytokine/chemokine levels, immune system deficiency and neuroinflammation [9]. However, at present the identification and temporal sequence of all the specific molecular mediators responsible for defective signalling between injured motor neurons and functionally impaired skeletal muscles remains a major challenge for research.

At the cellular level, the current evidence is that ALS is non-cell autonomous and damage to different cell phenotypes contributes to different phases of the disease [10]. Expression of mutated SOD1 (mSOD1) within motor neurons is a determining factor for disease onset, but also muscle cells are lately emerging as recipients and/or initiators of primary injury [11]. Nearest glial cells, especially astrocytes, microglia and Schwann cells [12, 13] then cope with mSOD1-mediated damage influencing disease progression. Finally, impairment at the blood-brain barrier leads to massive release of neurotoxic products into the CNS, thus bursting inflammation that tilts the already unstable balance of unhealthy motor neurons [14]. On this regard, recent reports have highlighted the presence in ALS patients and animal models of both neuroprotective and neurotoxic blood-derived immune cells, playing synergistic and critical functions mainly dependent on the different stages of the disease.

The role of a systemic immune activation in ALS is supported by abnormalities found directly in the blood and cerebrospinal fluid of patients, such as increased numbers of circulating lymphocytes, particularly $CD4^+$ T helper (Th) cells, $CD8^+$ cytotoxic T lymphocytes (CTL) and natural killer cells (NK), increased expression of MHC class II molecules on monocytes, together with higher levels of circulating inflammatory cytokines and chemokines [15, 16]. This is further corroborated in post-mortem studies of brain and spinal cord lesions from ALS patients, showing that the infiltration of macrophages, mast cells and T lymphocytes in close proximity to degenerating tissue, counterparts the activation of microglia/astrocytes [17, 18]. However, despite the active involvement that the immune system has demonstrated in ALS samples and tissues, whether neuroinflammation is a cause or a consequence of motor neuron dysfunction is still debated, as in general the exact mechanistic contributory role of the immune system to ALS pathogenesis.

Immune Responses in ALS

Results from ALS patients and pre-clinical studies strongly suggest that systemic innate and adaptive immune activation plays a central role in ALS pathogenesis [19]. However, a better understanding of the specific actions elicited by the different subtypes of immune cells is of utmost necessity. Indeed, cumulative evidence suggests that inflammatory cells mediate both protective and deleterious effects on motor neuron survival and that these actions vary as a function of disease progression.

The neurotoxic contribution of immune cells to ALS pathogenesis is well established. Memory cytotoxic $CD8^+$ CTL and NK cells are effector cells of the immune system, programmed to eliminate aberrant cells [20]. While an increased number of $CD8^+$ CTL and NK cells is observed in the blood and spinal cord of ALS patients at symptomatic stage [19, 21], it has been also reported that in ALS mice mSOD1 Th1 CTL proliferate to a greater extent and produce more interferon-γ (IFNγ) during the rapidly progressing phase, than Th1 lymphocytes isolated during the slowly progressing phase. Various mechanisms and different death pathways are then induced by CTL to contribute to motor neuron death in ALS. Among these, the activation of Fas (CD95) by its ligand FasL specifically commits motor neurons into a death program mediated by a caspase cascade [22]. Consistently, homozygous loss-of-function FasL mutation in mSOD1 mice reduces motor neurons loss and prolongs life expectancy [23]. Another cytotoxic mechanism of CTL-mediated damage to target cells is the perforin-granzyme system, where perforin is a pore forming protein allowing the entrance through the cell membrane of granzyme serine proteases responsible for caspase activation and cell death [24]. In serum of ALS patients, augmented levels of granzyme A and B isoforms have been found, although the functional significance of such an increase remains to the defined [25]. IFNγ, which is produced by CTL cells and by mSOD1 astrocytes and motor neurons also contributes to ALS pathogenesis, and intracerebroventricular delivery of neutralizing anti-IFNγ antibody has been shown to delay motor function decline in SOD1-G93A mice [26].

In parallel, the neuroprotective contribution of immune cells to ALS pathogenesis is also well established. The homeostatic phenomenon of protective immunity is crucial in the repair of damaged tissues and includes the clearance of cellular debris and the beneficial effects of cytokines and growth factors delivered directly by inflammatory cells to the sites of injury [27, 28]. Indeed, when SOD1-G93A mice are bred with mice lacking $CD4^+$ Th cells (an essential part of the cell communication system within the immune system), microglia switch toward an M1 inflammatory phenotype and disease progression accelerates, suggesting that $CD4^+$ Th cells are responsible for neuroprotection by suppressing the activation of cytotoxic microglia. Consistently, reconstitution of Th cells in bone marrow of SOD1-G93A mice lacking functional T and B cells prolongs animal survival and suppresses the activation of toxic M1 microglia [29]. Further studies have also shown that neuroprotection is mainly supported by $CD4^+CD25^+Foxp3^+$ Tregs that secrete interleukin-4 (IL-4), thus promoting protective M2 microglia, and by IL-4 secreting Th2 cells, that inhibit the neurotoxic Th1 response and IFNγ secretion. Also in this case, the transplantation of Tregs into ALS mice lacking functional T cells results in prolonged survival [30]. Interestingly, neuroprotective Tregs are increased in the peripheral blood of ALS patients during early stages, but they are thereafter decreased as the disease rapidly accelerates, thus following an inverse correlation trend [21, 30, 31]. Hence, Tregs and microglia improve the neuroprotective properties of the immune system during the stable disease phase, while a switch from a beneficial Tregs/M2 microglia to a deleterious Th1/M1 microglia response would define disease progression [32]. In addition, co-culture experiments have shown that Tregs suppress the expression of cytotoxic factors NADPHoxidase2 (NOX2) and inducible NOS (iNOS) from SOD1-G93A microglia, through IL-4 secretion, and inhibit the proliferation of

SOD1-G93A effector Th cells *via* the combined secretion of IL-4, IL-10, and TGF-β [33]. This is further sustained by a recent study documenting that adult microglia isolated from ALS mice at disease onset have an M2 phenotype and protect motor neurons, whereas microglia isolated from ALS mice at end-stage of disease possess an M1 phenotype and are neurotoxic, thus supporting the dual phenotypes of microglia and their functional transition during disease pathoprogression [34].

Also mast cells are lately emerging as main regulators with bi-functional role in both innate and adaptive immunity. As such, their dysregulation or impaired interaction with microglia can promote or amplify neuroinflammation occurring during neurodegenerative and neurocognitive disorders [35]. In the setting of autoimmunity, mast cells have a role in the initiation of the pathological immune response, by nurturing inflammation through the production of interleukin-6 (IL-6) and the shift of regulatory T cells (Tregs) into T helper (Th) Th17 cells producing interleukin 17 and interleukin 22. Furthermore, the mast cell-dependent interleukin-17A (IL-17A) mediated pathway, a signature of autoimmune diseases, seems to be critical in ALS, because both sALS and fALS subjects have apparently stronger concentration of the cytokine IL-17A in serum [36], and their spinal cord is found infiltrated in patchy fashion in gray matter by IL-17A-positive and tryptase-positive mast cells, in a way directly correlated to disease duration [36, 37]. Together with T cells and macrophages/microglia, mast cells are among the immune cells found infiltrated in ALS spinal cord and cortex, suggesting that inflammation in ALS is definitely based on both innate immune responses mediated by macrophages and mast cells, and adaptive immune responses sustained by T cells [17].

While further studies are definitely needed to dissect this complex interplay among peripheral and CNS-resident or -infiltrated immune cells, within the neuro-immune cross-talk, it is evident that the least lack of balance that might occur in any step of this complex cellular network will then become crucial for causing ALS insurgence and progression (Fig. **1**).

MICROGLIA REGULATION BY HISTAMINE

Microglia are antigen-presenting cells that express on their plasma membrane proteins of the MCH-II complex being, thereby, interaction partners with infiltrating T lymphocytes. They possess a wide range of characteristic immune cell receptors, such as chemokine, cytokine and complement-factor receptors. Being the intrinsic immune cells of brain and spinal cord, present in all regions of CNS, they are also able to respond to the signaling substances of the CNS, namely, neurotransmitters. Because of the diffusion of neurotransmitters in the entire extracellular space and not only concentrated at the synapses, it is now established that neurotransmitter receptors are also directly activated on microglia. Among these, here we will focus our attention particularly on the histamine receptors present on microglia.

Histamine [2-(4-imidazole) ethylamine] is a hydrophilic vasoactive amine involved in peripheral innate and adaptive immune responses and acting as a transmitter and signalling molecule that mediates central inflammation. As part of an immune response to fight foreign pathogens, histamine is produced by basophils and by mast cells found in nearby connective tissues. Unfortunately, when released inappropriately or in too high quantities, histamine is a potentially devastating substance. Histamine increases the permeability of the capillaries to white blood cells and some proteins, to allow them to engage pathogens in the infected tissues. It acts through four G-protein coupled receptors that cooperate by diverse intracellular pathways and have different therapeutic potentials, as they vary in expression, isoform distribution, signalling properties, and functions during diseases: H1 and H2 receptors are mostly excitatory; H3 receptors act as inhibitory auto- and hetero-receptors; the H4 receptors are expressed in human and rodent CNS, although they are not well defined yet in their function [38, 39]. While the behaviour of histamine as an inflammatory regulator in peripheral tissues is well-known, its role in central immune responses is still little investigated, particularly in microglial cells.

Back in 2001, Katoh and co-authors, have demonstrated that microglia in rat and mouse brain are the only other cell type, besides mast cells and neurons, capable of releasing histamine, among other inflammatory mediators [40]. Since then, the study of histamine function in microglia has become of a certain interest, especially because microglia-mediated neuroinflammation is now recognized to play a central role in chronic neurodegenerative conditions [41, 42]. For instance, it is established that histamine is implicated in the pathophysiology of multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE), by changing the permeability of blood brain barrier, bringing to an increase of infiltrated cells in CNS and subsequent neuroinflammation. Accordingly, EAE mice with the genetic deletion of H1/H2 receptors develop a less severe clinical disease course and decreased neuropathology with more preserved blood brain barrier permeability compared with wild-type (WT) mice [43]. On the other hand, there are results showing a protective role of histamine in MS and EAE [44]. While apparently contrasting, these results indicate that histamine surely plays a dual role in the pathogenesis of MS and EAE, and they also open a therapeutic potential perspective for histaminergic ligands in the treatment of autoimmune diseases [41].

Additional evidence suggests that abnormal histamine signalling in the brain may also be an important aspect in Gilles de la Tourette syndrome, Alzheimer and Parkinson's disease, neuropathic pain, sleep-wake disorders, addictive behaviours and, not last, eating behaviour [45-47]. In particular, numerous H3 receptor antagonists/inverse agonists have already entered Phase II–III clinical trials for these pathologies [48].

The first work to report a direct role of histamine in microglia describes the effect produced by histamine infusion in rat brain substantia nigra. Here histamine induces an acute inflammatory response characterized by a loss of immunolabelled glial fibrillary acidic protein (GFAP) positive astrocytes, by simultaneous activation of microglia and dopaminergic neuronal death. Thus, in the substantia nigra the inflammatory responses mediated by histamine seem fundamental for the pathological changes that cause dopaminergic neuronal damage after histamine infusion [49].

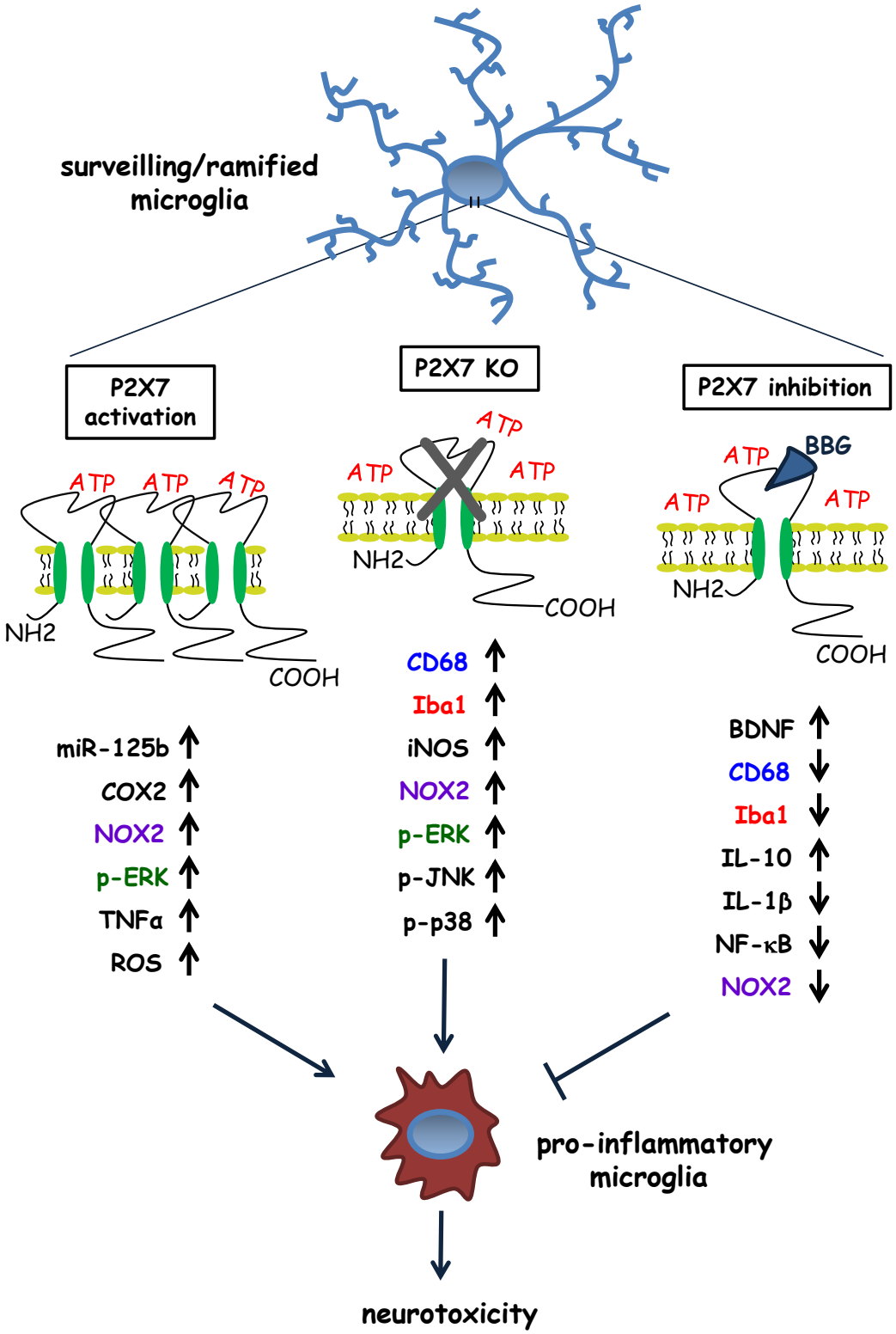

Fig. (1). P2X7 and microglia responses in ALS. In microglia from SOD1-G93A mice, P2X7 is overexpressed and its stimulation causes enhanced release of pro-inflammatory factors that are toxic to motor neurons. A similar picture of increased neuroinflammation is observed in SOD1-G93A mice lacking the *P2RX7* gene. On the contrary, a preclinical study with the P2X7 antagonist Brilliant Blue G (BBG) administered at late pre-onset phase of disease (~14 weeks) shows reduced microgliosis (Iba1 and CD68) and M1 microglia inflammatory markers (IL-1β, Nf-kB, NOX2), increased M2 markers (brain-derived neurotrophic factor and IL-10) and motor neuron survival, together with improved motor performance and general conditions in SOD1-G93A mice. COX2, cyclooxigenase 2; NOX2, NADPH oxidase 2; p-ERK1/2, phospho-ERK1/2; p-p38, phospho-p38; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α; p-JNK, phospho-JNK; iNOS, inducible nitric oxide synthase; BDNF, brain-derived neurotrophic factor; NF-κB, nuclear factor-kappaB; IL-1β, interleukin-1β; IL-10, interleukin-10.

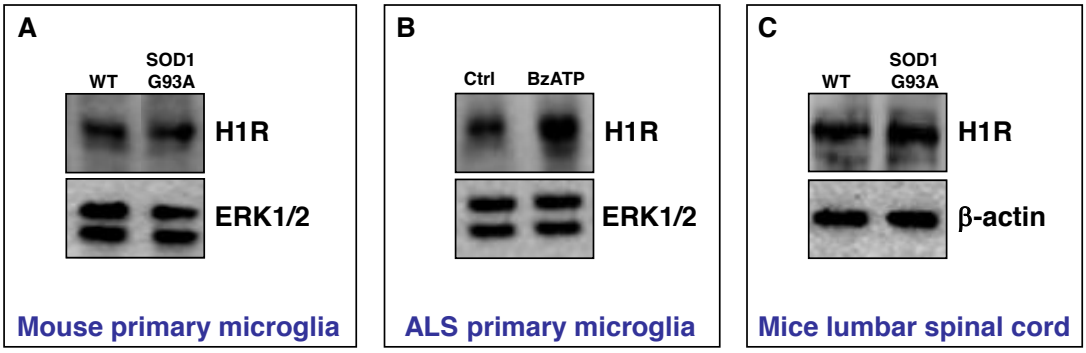

Fig. (2). H1 receptor in ALS. (**A**) Equal amount of total lysates from wild-type (WT) and SOD1-G93A primary microglia cells was subjected to western blotting with anti-H1 receptor (H1R). Anti-ERK1/2 was used for protein normalization. (**B**) SOD1-G93A microglia were treated with 100 μM 2'(3')-*O*-(4-Benzoylbenzoyl)adenosine-5'-triphosphate (BzATP) for 60 minutes and equal amount of total cell lysates was subjected to western blotting with anti-H1R. 2'(3')-*O*-(4-Benzoylbenzoyl)adenosine-5'-triphosphate induced an increase in H1R expression. Anti-ERK1/2 was used for protein normalization. (**C**) Equal amount of total lumbar spinal cord lysates from WT mice (6 months) and SOD1-G93A mice at end stage (~23 weeks) was subjected to western blotting with anti-H1R. Both WT and SOD1-G93A spinal cord show the presence of H1R subtype. Anti-β-actin was used for protein normalization.

Histamine microglia-related actions are established also in another disease in which neuroinflammation plays a crucial role, ischemia-reperfusion brain injury. Since facilitation of central histaminergic activity was previously shown to ameliorate reperfusion injury, the effects of post-ischemic administration of L-histidine, a precursor of histamine, were also evaluated. While ischemia induces a remarkable increase in the number of CD68-positive microglia cells, this effect is prevented by L-histidine through H2 receptors, suggesting that improvement of histaminergic system suppresses neuroinflammation after ischemic events [50].

After these encouraging *in vivo* results, the interest for the role of histamine in microglia further raised and in 2011 Seifert and collaborators studied the calcium responses of adult microglia/brain macrophages to a set of transmitter and hormones. The authors identified a subpopulation of primary rat microglial cells specifically sensitive to histamine that indeed respond with an increase in intracellular calcium originated by inositol-3-phosphate-dependent Ca^{2+} release from endoplasmic reticulum [51]. Moreover, the subpopulation of microglia sensitive to histamine increases in both adult and neonatal cells, after treatment with both lipopolysaccaride (LPS) and IFN-γ, thus demonstrating that microglia are more responsive to histamine in defined activation and developmental states [52]. Only in 2012 the group of Ferreira and co-authors have investigated the molecular mechanisms directly exerted by histamine and its receptors in microglia-induced inflammation, by studying microglia migration and release of inflammatory factors. Using a murine microglial cell line, they have mapped the presence of all histamine receptor subtypes H1, H2, H3 and H4, while they have established the presence of the H4 receptor in primary microglia cell cultures from rat cortex. The authors have then demonstrated that histamine plays a direct role in primary microglial cell motility, and in the release of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). These effects are apparently mediated by H4 receptor activation through a mechanism involving α5β1 integrins, p-38 and AKT signalling pathways. Interestingly, while histamine can induce these effects alone, when primary microglia are challenged in the simultaneous presence of LPS, histamine then inhibits LPS-stimulated migration *via* H4 activation. Histamine and H4 receptor agonists also inhibit LPS-induced IL-1β release in both microglia cell line and hippocampal organotypic slice cultures. This suggests a dual role of histamine in the modulation of microglial inflammatory responses, indicating that while histamine *per se* triggers microglia motility, in the presence of a toxic stimulus such as LPS, it instead hampers microglia activation [53]. These results thus demonstrate that the microglial responses to histamine are subordinated to the particular environmental context in which histamine is acting at each time.

Further work has then established that all four histamine receptors are present in primary rat microglial cultures and, interestingly, the expression of H1 and H4 receptor subtypes is selectively up-regulated by histamine in a dose-dependent manner. In this context, histamine *via* H1 and H4 receptors directly stimulates microglia activation, induces release of pro-inflammatory factors such as TNF-α and IL-6, as well as MAPK, PI3K/AKT and nuclear factor-κB (NF-κB) signalling pathways, together with loss of mitochondrial membrane potential [54]. In microglial cells from neonatal rat brain, the effects of histamine on the increased production and release of another inflammatory factor, nitric oxide (NO), has shown that histamine significantly increases the expression of iNOS, suggesting that histamine acts as a NO-regulating factor by inducing iNOS expression [42].

All these studies prove that microglial cells specifically express functional histamine receptors that might therefore become important partners in the intercellular communication system of the CNS. As a consequence, they open up a new scenario in the study of histamine effects related to the treatment of CNS diseases accompanied by microglia-mediated neuroinflammation.

MICROGLIA MODULATION BY P2X7 RECEPTOR IN ALS

Among the most common factors released in addition to histamine during acute and chronic pathological insults to

the nervous system, extracellular purines and pyrimidines can target the many different cell phenotypes participating to a neuroinflammatory response. Moreover, they can directly initiate and/or propagate neurodegeneration and neuroinflammation, as well as behave as negative modulators in this process [55]. Particularly in ALS, a wide range of factors have been suggested to induce neuroinflammatory responses and to mediate the detrimental or beneficial effects that glial cells exert on motor neurons. Among these, purinergic ligands are assuming an increasingly importance being key mediators of intercellular communication. Indeed, microglia, astrocytes and also degenerating neurons can release and simultaneously respond to extracellular ATP and other purine/pyrimidine nucleotides binding to specific ionotropic (P2X) and metabotropic (P2Y) receptors. Particularly the P2X7 subtype is an ATP-gated cation channel that is widely expressed in cells of the immune system, where it has been implicated in the processing and release of cytokines such as IL-1β, as well as in the initiation of cell death mediated by both apoptotic and necrotic pathways. Thus, since its discovery in lymphocytes and mast cells [56, 57], the P2X7 has been proposed to function as a major regulator of inflammation and immunity [58, 59].

In the nervous system, P2X7 is abundantly present on microglia and astrocytes and the receptor is up-regulated around amyloid β-peptide plaques in transgenic mouse models of Alzheimer disease, in spinal cord following injury, in amyotrophic lateral sclerosis spinal cord, and in cerebral cortex after ischemia and multiple sclerosis [60]. Utilizing neuron/microglia co-cultures as an *in vitro* model system for neuroinflammation, in our previous work we have demonstrated that P2X7 receptor up-regulation and activation on microglia appears necessary for microglial morphological transition, reactive oxygen species (ROS) production and cell-mediated injury to neurons [61]. Moreover, the receptor is emerging as a "gene modifier" in ALS [62]. Indeed, P2X7 is up-regulated in ALS spinal cord microglia in human [63] and rat at advanced stages of disease [64]. In P2X7$^{-/-}$/SOD1-G93A mice, we have shown that the clinical onset of ALS is anticipated and the disease progression worsened in both male and female genders. In addition, genetic deletion has resulted in increased motor neuron loss, microgliosis and astrogliosis in lumbar spinal cord, activation of NOX2, iNOS and phospho-ERK1/2 (p-ERK1/2) kinase, thus suggesting an unanticipated protective role of P2X7 [65]. However, *in vitro* activation of P2X7 deregulates inflammatory microRNAs among which miR-125b by interfering with the STAT3 pathway and determining increased TNFα transcription [66]. Moreover, P2X7 activation exacerbates pro-inflammatory responses such as NOX2 activity, ROS production, TNF-α, cyclooxygenase-2 (COX-2) and MAPKs levels in ALS-microglia, and exerts toxic effects towards SOD1-G93A neuronal cells [61, 67]. Similar *in vitro* effects of P2X7 are also extended to astrocytes [68]. Consistently with these results, pharmacological treatment of SOD1-G93A mice with the preferential P2X7 antagonist Brilliant Blue G (BBG) initiated at late pre-onset significantly delays onset and improves mice general conditions and motor performance in both males and females. This is obtained without affecting survival, but with a concomitant decrease of microgliosis, marked modulation of inflammatory markers such as NF-κB, NOX2, IL-1β, IL-10 and brain-derived neurotrophic factor and, finally, enhanced survival of motor neurons in L3-L5 ventral spinal cord. In particular, the expression of pro-inflammatory M1 microglia markers is reduced, with concomitant increase of anti-inflammatory M2 markers [69]. A key feature that emerged from these studies is the time-dependency of BBG in showing protective effects. Differently from the worsened SOD1-G93A phenotype obtained with the genetic ablation of P2X7 [65], the treatment with the P2X7 antagonist BBG started at late pre-onset, but not at pre-symptomatic phases of the disease or after onset, is indeed shown to moderate ALS progression and improve motor neuron survival. On the whole, these results would outline a potential therapeutic window of intervention against ALS, consistent with the decisive switch that microglia undergo from a beneficial M2 to a detrimental M1 phenotype, and perhaps corresponding to the time when P2X7 might become critical in modulating neuroinflammatory pathways in ALS [69]. Although recent studies prove that SOD1-G93A microglia exhibits a disease-specific transition not classically corresponding to a typical M1 or M2 phenotype [70], this switch likely occurs when disease progresses [30, 32] and treatment with BBG becomes effective [69]. In addition to the dual role played by neuroinflammation and microglia in ALS [71], these data thus suggest that also P2X7 might behave as a dual modifier in the disease, consistently with toxic versus trophic P2X7 actions previously demonstrated in surveilling versus activated microglia, and moreover dependent on the different levels of ATP release [72, 73].

Finally, these data once again reinforce that ALS is a multi-systemic pathology against which a single therapeutic strategy might not be sufficient, while the multiple modulation of different biological pathways might result successful to counteract the deleterious outcome of the disease.

H1 RECEPTOR IN ALS MOUSE MODEL

Interactions between extracellular ATP and several different neurotransmitters are already proven to play key roles in various CNS functions and in health and disease conditions [74]. However, very little is still recognised particularly about histaminergic and purinergic co-transmission. We know for instance that in peripheral systems, extracellular ATP modulates histamine release from mast cells [75] and, on the other hand, histamine induces ATP outflow *via* pannexin-1 hemichannels from fibroblasts [76]. In addition to this mutual regulation of transmitter release, a reciprocal modulation of receptor expression and/or activity might also occur. In CNS, microglia consist of heterogeneous populations with respect to sensitivity to neuropeptides and neurotransmitters [77], and a subset of ATP-responding microglial cells are indeed known to simultaneously react to histamine and to become even more responsive after induction of a pro-inflammatory phenotype [51, 52]. Moreover, in human macrophages, a marked potentiation of P2X7 activity is induced by the H1 antihistaminic clemastine that sensitizes the receptor to lower ATP concentrations, by acting as a positive allosteric modulator [78, 79]. Corroborating these earliest results, we

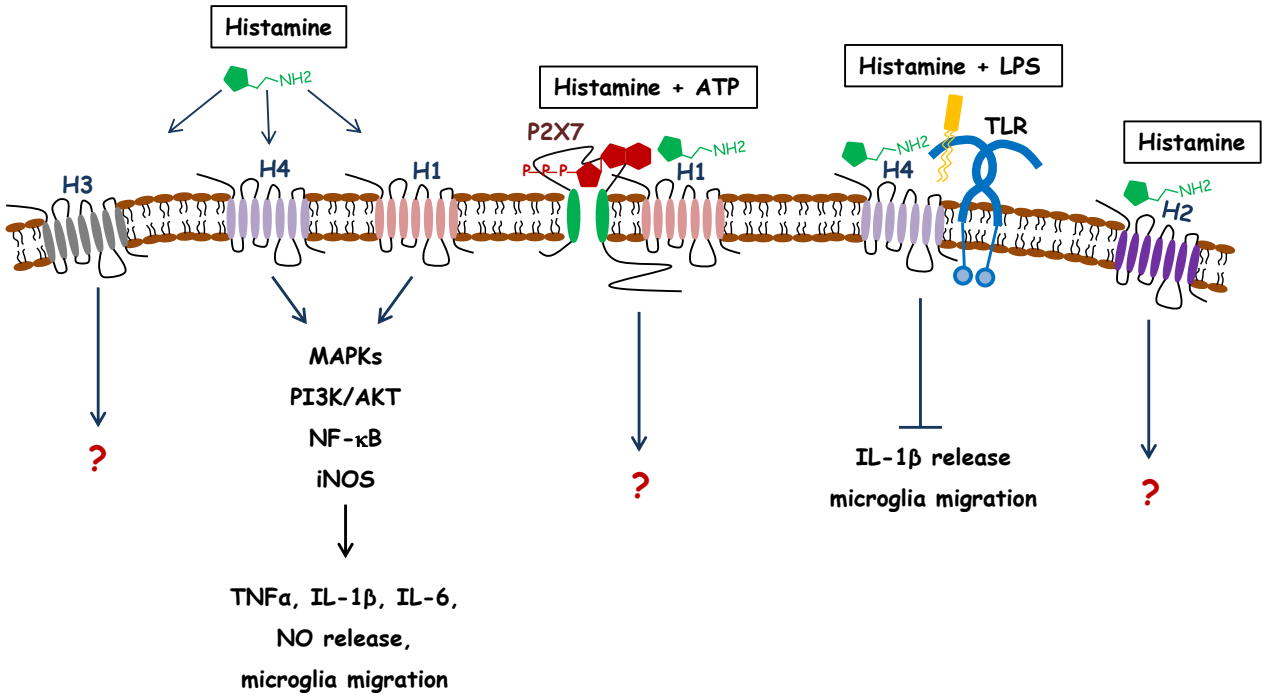

Fig. (3). Histamine and histamine receptors in the modulation of microglial inflammatory responses. Microglial cells constitutively express all known histamine receptors H1, H2, H3 and H4. Histamine *per se* acts primarily as a pro-inflammatory agent through H1 and H4 receptors by inducing TNFα, IL-1β, IL-6, NO release and microglia migration through MAPKs, PI3K/AKT and NF-kB pathways activation and iNOS induction. However, in the LPS-induced inflammatory context, histamine has an inhibitory action on microglia migration and release of IL-1β, through H4 receptor. The role of H2 and H3 in microglia cells has not been exploited yet. Moreover, we have recently found that activation of P2X7 receptor up regulates H1 receptor, thus suggesting a potential cooperation among ATP and histamine signalling. TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; NF-κB, nuclear factor-kappaB.

established that an interaction between histaminergic and purinergic signalling might also occur in ALS microglia, and that histamine might thus play a key role in ALS. Indeed, the expression of the pro-inflammatory H1 receptor in primary microglia from ALS mice is increased by the preferential P2X7 agonist 2'(3')-*O*-(4-Benzoylbenzoyl)adenosine-5'-triphosphate (Fig. **2A**, **B**), and the presence of H1 receptor is established in lumbar spinal cord of SOD1-G93A mice at end stage of disease, although remaining unchanged with respect to healthy mice (Fig. **2C**). Interestingly, a chronic *in vivo* treatment in ALS mice with the CNS-penetrant H1 receptor antagonist clemastine down regulates P2X7 in lumbar spinal cord, while reducing microgliosis, modulating microglia-related inflammatory genes and enhancing motor neuron survival [80]. Finally clemastine *in vitro* exerts a dual action on ALS microglia-mediated inflammatory pathways, by activating M2-associated markers and simultaneous inhibiting M1 markers [80].

These results clearly suggest a level of potential cooperation between purinergic and histaminergic systems in ALS microglia, furthermore highlighting a novel approach for dissecting ALS pathology and exploiting the modulation of ALS-related neuroinflammation. In addition to the other neuroinflammatory and neurodegenerative conditions where histamine modulation has already been described, further studies are required in order to define to which extent the histaminergic transmission might affect the ALS disease.

CONCLUSION AND FUTURE DIRECTIONS

ALS patients display evidence of chronic inflammation as demonstrated by diffuse infiltration of inflammatory macrophages/microglia, and patchy infiltration of mast cells, dendritic and T cells, into grey matter spinal cord [17, 36, 37]. Because microglia, mast cells and neurons in rat and mouse brain are the only cell types capable of releasing histamine [40], and recent work has highlighted the possibility that microglia might contribute to the exacerbation of neurodegenerative diseases by modulating the activation of mast cells that normally control neuronal function [81, 82], we have gathered here some example of how microglia can directly respond to histamine. Upon mast cell degranulation occurring abundantly at sites of injury, the released histamine can in fact act back on microglia histamine receptors that cooperate on multiple arms of the immune response. Because very little is currently known about the role of histamine modulation particularly in microglia involved in neuroinflammatory diseases among which ALS, the purpose of this review has been to highlight the histaminergic system as a new prospective candidate able to cope with inflammatory mechanisms particularly in ALS, and perhaps in a concerted action with the already established P2X7-mediated signalling (Fig. **3**). In light of the dynamic functional changes of microglia and immune cells discussed above in the context of ALS, attempts to target neuroinflammation through histamine and extracellular ATP

modulation might constitute a novel path for decoding the multi-systemic nature of ALS pathogenesis. Future studies will now have to prove how the new player histamine will possibly get along with microglia-sustained inflammation in ALS.

LIST OF ABBREVIATIONS

ALS	=	Amyotrophic Lateral Sclerosis
BBG	=	Brilliant Blue G
COX2	=	Cyclooxigenase 2
CTL	=	Cytotoxic T Lymphocytes
EAE	=	Experimental Autoimmune Encephalomyelitis
fALS	=	Familiar Amyotrophic Lateral Sclerosis
GFAP	=	Glial Fibrillary Acidic Protein
IFNγ	=	Interferon-γ
IL	=	Interleukin
iNOS	=	Inducible Nitric Oxide Synthase
LPS	=	Lipopolysaccaride
miRNA	=	microRNA
MS	=	Multiple Sclerosis
mSOD1	=	Mutated SOD1
NF-κB	=	Nuclear Factor-KappaB
NK	=	Natural Killer Cells
NO	=	Nitric Oxide
NOX2	=	NADPH Oxidase 2
p-ERK1/2	=	Phospho-ERK1/2
p-JNK	=	Phospho-JNK
p-p38	=	Phospho-p38
ROS	=	Reactive Oxygen Species
sALS	=	Sporadic Amyotrophic Lateral Sclerosis
SOD	=	Superoxide Dismutase
Th	=	Helper T Lymphocytes
TNF-α	=	Tumor Necrosis Factor-α
Tregs	=	Regulatory T Lymphocytes
WT	=	Wild-Type

CONFLICT OF INTEREST

The Authors confirm that this article content has no conflict of interest.

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