Microglia activation as a therapeutic target in multiple system atrophy: The timing, the good and the bad

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Microglial activation has turned out as one of the key factors during the disease process in neurodegenerative disorders. It has become clear that activated microglia contribute to the pathogenesis and progression of symptoms in a complex and multifaceted way, carrying out different functions which can affect the disease process in a positive or negative manner. We focus on neuroinflammation as a player in the pathogenesis of multiple system atrophy (MSA) – a rare neurodegenerative disorder characterized by α-synuclein accumulation in oligodendrocytes and selective neuronal loss. We discuss here recent findings in a transgenic mouse model of MSA supporting the notion of the bipolar effects of microglial activation linked to toxic pro-inflammatory signalling parallel to the neuroprotective α-synuclein clearance by microglia. Furthermore, we discuss microglial activation as a candidate therapeutic target for MSA and summarize preclinical data on the neuroprotective efficacy of myeloperoxidase inhibition dependent on the initiation of the treatment in relation to the clinical onset of the disease.

Keywords: microglial activation; neurodegeneration; neuroinflammation; myeloperoxidase; TLR4; synuclein

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Microglia have become one of the most attractive research targets in the last decades. Far away from being just the “glue” that keeps neurons together, or a mere support for them, these cells have been widely shown to have a pivotal role in the central nervous system (CNS). They represent the resident macrophages in the brain, continuously surveying the surrounding microenvironment (in their so called “resting state”) and reacting in case of infection or tissue injury (becoming “activated”); furthermore, it has been recently shown that they are also involved in synaptic homeostasis and plasticity, suggesting a broader role in the maintenance of the CNS and in some more specific brain activities, such as learning [1].

One of the main characteristics that render microglial cells so interesting in the field of neurodegeneration is their role in triggering and maintaining neuroinflammation, upon their activation. On the one hand, microglial response is of vital importance to protect the brain against any kind of threat for its health, being most effective in its acute form; on the other hand, the neuroinflammatory process can get detrimental itself, if dysregulated, turning in a chronic, auto-propagating event [2]. This condition is a common feature of all neurodegenerative diseases, and that is why microglial activation emerges as such an intriguing actor in this...
As IL-10 and IL-4), and in the restoration of a homeostatic, which is an anti-inflammatory one, is involved in the antigen presentation. The alternative activation form instead, is the elimination of the host element, with subsequent and nitrogen species; the main aim of microglia in this phase downregulation of the inflammatory process, principally like TNFα, IL-1β, IL-6 [4], chemokines and reactive oxygen and nitrogen species; the main aim of microglia in this phase is the elimination of the host element, with subsequent antigen presentation. The alternative activation form instead, which is an anti-inflammatory one, is involved in the downregulation of the inflammatory process, principally through the production of anti-inflammatory cytokines (such as IL-10 and IL-4), and in the restoration of a homeostatic, healthy condition, through debris clearance, wound healing and matrix deposition [3]. It could be that the normal balance between these two activation forms, generally present during acute inflammatory events, gets lost during chronic neuroinflammation, in a sort of vicious cycle. The main activation form in this case would be the M1, leading to an exacerbation of the damage derived by the continuous production of pro-inflammatory cytokines. In parallel the detrimental effect of chronic microglial activation may relate to the lack of positive effects provided by the M2 phenotype, including deficiency of anti-inflammatory cytokines and neurotrophic factors [3].

Other classifications of activated microglia have also been proposed. For instance, Sanchez-Guajardo and colleagues [5] proposed the definition of four subtypes of microglial activation profile (A, B, C and D) according to the cell morphology. In particular, Mac1 positive cells (microglia) have been distinguished based on length and thickness of their processes and the appearance of cell body and nucleus [5]. This classification ranges from resting microglia (type A cells, with thin processes and little branching) to fully activated cells (type D, presenting with an amoeboid shape, identical to peripheral macrophages), passing through stage B (very ramified) and C (reminiscent of antigen presenting cells, processes become shorter and thicker). Sanchez-Guajardo and colleagues have applied this classification in their study on a Parkinson’s disease model, observing activated microglia of specific subtypes at different time points. Interestingly, the results of this study are indicative of a specific, targeted response of microglial cells to various disease stages, which suggests the importance of getting a better insight in the timing-mechanisms of microglial activation during neurodegenerative processes.

When talking about microglial activation, one has to keep in mind that this is a highly dynamic process, actually involving many stages and forms, from both the morphologic and the functional point of view. The most diffuse and overall accepted one is the M1/M2 classification of microglial activation, where M1 represents the so-called “classical activation” and M2 the “alternative activation” [3]. The first one, which can be defined as a pro-inflammatory form, is characterized by the production and release of pro-inflammatory mediators, such as inflammatory cytokines like TNFα, IL-1β, IL-6 [4], chemokines and reactive oxygen and nitrogen species; the main aim of microglia in this phase is the elimination of the host element, with subsequent antigen presentation. The alternative activation form instead, which is an anti-inflammatory one, is involved in the downregulation of the inflammatory process, principally through the production of anti-inflammatory cytokines (such as IL-10 and IL-4), and in the restoration of a homeostatic, healthy condition, through debris clearance, wound healing and matrix deposition [3]. It could be that the normal balance between these two activation forms, generally present during acute inflammatory events, gets lost during chronic neuroinflammation, in a sort of vicious cycle. The main activation form in this case would be the M1, leading to an exacerbation of the damage derived by the continuous production of pro-inflammatory cytokines. In parallel the detrimental effect of chronic microglial activation may relate to the lack of positive effects provided by the M2 phenotype, including deficiency of anti-inflammatory cytokines and neurotrophic factors [3].

As in all neurodegenerative disorders, also in Multiple System Atrophy (MSA) microglial activation has been described and identified as one of the main features of the disease process [6-11]. MSA is a progressive neurodegenerative disorder distinguished by parkinsonism, cerebellar ataxia and autonomic failure [6]. The histopathological characteristic of the disease is the presence of glial cytoplasmic inclusions (GCIs) -aggregates of αSynuclein (αSyn) within oligodendrocytes- that lead to a specific dysfunctional phenotype. As a consequence of this, the neurotrophic support that oligodendrocytes normally provide to neurons gets lost, and this initiates the neurodegenerative process [7]. The entire pathological process is accompanied by a chronic neuroinflammatory state, in which microglia emerge to be a major player [8]. In line with the concept of the dual role that these cells play in neurodegenerative conditions, our group has collected various results and observations in the last years, showing both neuroprotective and detrimental effects of microglial activation in MSA [9]. In a study of 2007 on transgenic mice, characterized by overexpression of human αSyn in oligodendroglia under the proteolipid protein (PLP) promoter (an MSA model), we found prominent microglial activation in the Substantia Nigra (SN) of aged transgenic animals if compared with younger ones, which could not be observed in the control C57Bl/6 mice [9]. We also demonstrated an increase in microglia cell numbers in SN and striatum of the PLP-αSyn mice, whereas no significant changes were detectable in the control animals. Another proof of the neuroinflammatory state in this model was the presence and increase with aging of inducible nitric oxide synthase (iNOS, an enzyme involved in the production of reactive nitrogen species). In the same study, we were interested to determine if there was a correlation between microglial activation and neurodegeneration in the PLP-αSyn model. Therefore, we treated young MSA mice with minocycline (a tetracycline with anti-neuroinflammatory effects) initiating the therapy before the onset of microglial activation and neurodegeneration for a period of two months [9-11]. We observed a suppression of microglial activation and reduced iNOS levels in PLP-αSyn mice exposed to early-start treatment with minocycline as opposed to those receiving saline. The early inhibition of neuroinflammation in MSA mice was associated with prevention of loss of dopaminergic neurons in SN and of dopaminergic terminals in the striatum,
thus confirming the role of microglial activation as inductor of neurodegeneration in the early stages of MSA.

In a following work we identified myeloperoxidase (MPO), a heme enzyme involved in the production of oxidizing species in phagocytic cells, to be up-regulated in microglial cells of MSA brains as well as in the brains of PLP-αSyn mice [12]. There is growing evidence for the role of MPO in promoting oxidative stress during inflammation; indeed, it uses hydrogen peroxide to oxidize several substrates to cytotoxic species, which can activate stress responses within the cells [13]. Its presence and contribution to disease development has already been shown in several acute and chronic inflammatory conditions, including stroke, Alzheimer’s disease and Parkinson’s disease [14, 15]. We demonstrated the expression of MPO also in MSA, both in patients’ post-mortem brain tissue and in the mouse model, with microglia being the main cell type producing it. MPO immunoreactivity was shown in MSA post-mortem mesencephalon, basal ganglia and white matter tracts, while no significant levels of the enzyme were detected in the same brain areas in control subjects [12]. At the cellular level, MPO was expressed by HLA-DR positive activated microglia, confirming the putative involvement of these cells in their reactive form during disease progression. Comparable results were found in the PLP-αSyn transgenic mice [12]. Therefore we tested the neuroprotective efficacy of a myeloperoxidase inhibitor (MPO-i) on PLP-αSyn transgenic mice. To exacerbate the MSA phenotype in the model and trigger the complete MSA-like pathology, including striatonigral degeneration (SND) and olivopontocerebellar atrophy (OPCA), transgenic mice were intoxicated with 3-nitropropionic acid (3-NP) [10, 12, 16]. 3-NP triggers mitochondrial dysfunction, that is suggested to play a significant role in MSA pathogenesis and has been previously shown to initiate SND and OPCA in PLP-αSyn mice [16, 17]. We demonstrated that early treatment with MPO-i, starting one week before the induction of SND and OPCA by 3-NP (i.e. before the “clinical onset” of disease), had beneficial effects on the motor dysfunction and was linked to neuroprotection and attenuation of neuronal loss in the disease sensitive brain regions (Figure 1A). Furthermore, measuring the relative optical density of CD11b immunoreactive cells, we confirmed a decrease of microglial activation in SNc and striatum associated with the MPO-i treatment. Interestingly, the reduced level of microglial activation in SN and striatum of MSA mice treated with MPO-i correlated with a significant reduction of αSyn nitration and aggregate formation in the same regions, indicating an important interplay between neuroinflammation and α-synucleinopathy in the disease process of MSA-like neurodegeneration.

The overall data collected with this study were indicative of an important neuroprotective potential for the MPO-i treatment. However, MSA is a disease that is hardly detectable at early stages in patients. Considering the need for a translational validity of the experimental data to humans, we undertook a new project, in order to test the therapeutic potency of MPO-i in a model of advanced MSA [18]. This time, the treatment with MPO-i (or vehicle) of transgenic PLP-αSyn mice was started only after the intoxication with 3-NP; when neuronal (fading blue) and oligodendroglial degeneration (fading orange) were already present, it was insufficient to provide neuroprotection and functional improvement, in spite of microglial suppression and reduction of α-synuclein load [18].
The fact that microglial activation was decreased also in this late experimental paradigm confirmed the potential efficacy of MPO-i to exert effects on neuroinflammatory responses in MSA; however, the even more important message that arises from these data is that the right timing of treatment initiation when targeting neuroinflammatory responses plays a key role in providing a successful disease modifying approach. In spite of the reduced microglial activation and the decrease of αSyn nitration and GCI formation upon MPO-i therapy in MSA mice, the late initiation of the treatment after the onset of neuronal loss and respective motor symptoms was inefficient to provide neuronal rescue.

Another feature characterizing microglial activation in MSA is the up-regulation of toll-like receptor 4 (TLR4) that we reported both at post-mortem examination in MSA brains and in the transgenic PLP-αSyn mouse\[^9,10\]. TLR4 belongs to the family of TLRs, highly conserved molecular pattern recognition receptors which play a key role in the innate immune system\[^10\]. TLRs’ up-regulation in MSA cases was confirmed in 2013 by Brudek et al.\[^20\] who demonstrated by RT-PCR increased mRNA expression for TLR3, TLR4 and TLR5 in substantia nigra, striatum, cerebral cortex, and nucleus dentatus, elevated mRNA TLR1 in substantia nigra, and striatum, and elevated mRNA TLR9 in cerebellum. The question was what is the role of TLRs in the puzzle of MSA disease process? Our group created a TLR4 deficient murine model of MSA, mating TLR4 knock-out mice with PLP-αSyn transgenic animals\[^21\]. We used TLR4 expressing PLP-αSyn mice as a control, and performed the tests at six months of age. What we found was a worse motor outcome in the TLR4-deficient group, giving a first indication of the beneficial role that the receptor might have in MSA. We further observed an increased, strong loss of dopaminergic neurons in SNc in the TLR4-deficient/αSyn mice, more prominent than in the control TLR4-positive/αSyn group\[^21\].
It appeared that TLR4 mediated at least partly the clearance of extracellular αSyn by microglia in vivo, suggesting an innate neuroprotective mechanism against the typical accumulation of this protein in the disease. We extended these studies by in vitro experiments to confirm the phagocytic capacity of primary TLR4-positive or -deficient microglia incubated with recombinant αSyn. The latter showed an evident impairment in phagocytizing the protein, if compared with the first one. Only 2% of the TLR4-deficient microglial cells were active in the αSyn-engulfment activity, against the 40% of the TLR4-positive microglia. These data suggested a protective role for microglia through TLR4-mediated clearance of extracellular αSyn. Indeed, a similar mechanism has been demonstrated in a scrapie model, reporting that the ablation of TLR4 causes a reduced immune response to the abnormal prion protein PrPSc and a higher grade of its accumulation in comparison with the control mice.

Taken together, all this information substantiates the complexity of microglia and of their response, in particular in conditions of chronic neuroinflammation: these cells cannot be restricted to a definition of good or bad, as the multifaceted way they get activated and contribute to inflammatory processes and diseases makes it impossible to consider them as static players in the intricate mechanism of neurodegenerative disorders. The different dynamic profiles of microglial activation and their timing in the progression of the neurodegenerative process in MSA seem to be critical in identifying the correct therapeutic window to target microglial activation for disease modification (Figure 2). Hence, a major focus on microglial activation is needed to understand how the various forms of activated microglia correlate with different disease stages, as this knowledge could supply a powerful tool for a targeted therapeutic approach for neurodegenerative diseases.

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Authors' contributions

VR, first draft and final approval of the manuscript. NS, concept, corrections and final approval of the manuscript.

References


