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Mesenchymal stem cells for the treatment of neurological diseases: Immunoregulation beyond neuroprotection

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ABSTRACT

An inflammatory response is often observed in neurological diseases, being characterized sometimes by activation of adaptive cells (T and B lymphocytes) and, almost inexorably, of cells of the innate immunity (microglial cells, macrophages). Mesenchymal stromal/stem cells represent a promising therapeutic approach for the treatment of intractable neurological diseases given the possibility that they affect neurodegeneration both directly and indirectly, through their potent immunomodulatory effect. Here we will review the evidence, mostly deriving from preclinical studies, that MSC, beyond their ability to foster neurorepair, can ameliorate neurodegenerative diseases through their effect on associated immune responses.

1. Introduction

The very recent discovery of a lymphatic drainage of the CNS was the coup de grace for the classical view of the brain as an immune privileged site [1–2]. Indeed, long before this discovery, the interplay between the central nervous system (CNS) and the immune system was known to be so close that the latter had been labeled "the sixth sense" [3]. Therefore, it comes as no surprise that perhaps all chronic diseases of the CNS are associated to an inflammatory response, which is believed to be the main driver of disease in the autoimmune condition, multiple sclerosis (MS), but is also observed in conditions classically labeled as neurodegenerative. In MS, the immune response has in most cases a biphasic pattern, with an initial phase characterized by an influx of mainly lymphocytes from peripheral blood to restricted sites of the CNS (plaques) together with activation of innate cells, such as microglial cells and macrophages and a second phase of diffuse inflammation involving both the white and gray matter of the CNS dominated by microglia and macrophages, usually associated with the progressive phase of MS. Interestingly, progressive MS is defined by the gain of irreversible disability, which is the main feature of primarily degenerative diseases (albeit with very distinct clinical features depending on the specific diseases), whose immune pathology is similarly dominated by compartmentalized inflammation involving cells of the innate immunity.

Innate inflammation is dominated by microglial cells, which are the resident immune cells of the CNS where they migrate at early stages of development, and by macrophages. Resting microglial cells display a ramified shape and are thought to have an homeostatic role and contribute to CNS immunosurveillance (although they are not able to migrate to lymph nodes to initiate immune responses) [4]. Loss of inhibitory signals from neurons or inflammatory and toxic cues within the CNS activate microglial cells that acquire an amoeboid shape, increase their phagocytic capability, release proinflammatory molecules and can give rise to macrophages that are morphologically indistinguishable from those derived by monocytes migrated from peripheral tissues through the inflamed blood brain barrier [4]. The role of activated microglial cells/macrophages in neuroinflammation has been classically defined as detrimental (classically activated, "M1" cells releasing IL-12, IL-1β, TNF-α, interferon-IFN-γ and nitric oxide, that lead to bystander tissue damage resulting in neurotoxicity) or beneficial (alternatively activated, "M2 cells" producing larger amounts of IL-10, IGF-1 and IL-4, that have phagocytic activity and neuroprotective features) [5]. While we acknowledge that this classification is likely too simplistic, since microglia display a spectrum of phenotypes in parallel with a continuum of activation [6], we will maintain it in this review for convenience. Adaptive cells entering the CNS in MS are in general regarded as detrimental, although inflammation-resolving T cells are also involved in the repair process [7]. Similarly, cells of the innate immunity associated

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to neurodegeneration have been described both as pathogenic and as an attempt of neurorepair [6,8]

Mesenchymal stromal cells (MSCs) often defined as mesenchymal stem cells, are multipotent cells of stromal lineage that can be isolated from the bone marrow (BM), adipose tissue, umbilical cord and several other tissues, and have been investigated as a potential alternative therapeutic approach for untractable neurological diseases for well over a decade [9]. The rationale for using MSCs in chronic untractable diseases of the CNS partly relies on their possible neuroprotective effect, with the hope that MSC may slow or halt the development of irreversible disability [10]. However, MSCs have potent effects on immune responses that may crucially contribute to their beneficial effect in neurological diseases. In 2002, Di Nicola et al. demonstrated for the first time that MSCs affect immune responses, followed shortly after by others [11–12]. Following these seminal studies, the immunomodulatory effects of MSCs on circulating adaptive, as well as innate, immune cells have been extensively studied [13–29]. In vitro, MSCs affect also the behavior of CNS cells such as microglial cells, and particularly inhibit microglial proliferation and their release of proinflammatory molecules; more specifically, MSCs induce the release of neuroprotective molecules by microglia and increase their phagocytic capability through the release of soluble factors [30–35]. Similarly, MSCs are able to skew the phenotype of macrophages from M1 to M2 type [36,37].

Therefore MSCs, with their potential multifactorial effect, appear as a promising alternative/adjunctive therapy for neurological diseases. In this review, we will discuss the impact of MSC treatment on immune responses associated to MS, a paradigmatic autoimmune disease of the CNS, amyotrophic lateral sclerosis (ALS) a classical neurodegenerative disease of the motor neurons, Alzheimer's disease (AD), a chronic progressive disease associated to intra- and extracellular deposition of toxic proteins, traumatic injury of the spinal cord (spinal cord injury, SCI) as an example of a disease of the CNS where neurodegeneration is caused by an external cause, and ischemic stroke, a very common cause of neurological impairment caused by loss of blood supply to brain areas.

As we will review, routes of administration have included local delivery (intralesional, intraventricular) chosen most often for primarily degenerative diseases, and in some cases in the animal model of MS, experimental autoimmune encephalomyelitis (EAE) and in patients with MS, and peripheral administration (intravenous -iv-, intraperitoneal). Local treatment was chosen initially with the aim of promoting engraftment of MSCs into diseased tissues, but it is now widely accepted that the engraftment of MSCs is scarce and that they exert their effect mainly by releasing molecules that protect the tissues and modulate the immune responses. Indeed, studies in EAE have revealed that iv administration affects peripheral and central immune responses and is equal to local delivery in terms of efficacy [38]. Moreover, pre-clinical studies have indicated that xenogeneic (human) MSCs are effective [39], despite the evidence of immune rejection [40], again arguing against the need of engraftment for their function. Indeed, the view that iv injection affects only peripheral immune responses is likely too simplistic: after iv transplantation, MSC are mainly trapped into the lungs [41–42], which harbor a complex mucosal immune system including macrophages, dendritic cells (DC) and lymphocytes [43]. The interaction between MSCs and the lung immune cells might trigger the properties of MSCs, as shown for instance by a study showing that MSCs that localized in the lungs after iv injections released the anti-inflammatory molecule tumor necrosis factor α-stimulating gene-6, but those which were captured by the spleen did not [44] (reviewed in [45]). Further studies on the immunological events occurring within the lungs after MSC injection are needed to better understand the mechanisms of MSC effects on the immune system.

In this article, we will review the evidence regarding the effect of MSC treatment on immune responses and inflammation associated to neurological diseases. Despite the fact that some clinical trials with MSCs for human neurological diseases have already been conducted and published [10], studies on their immunomodulatory mechanism of action were performed almost exclusively in animal models, in particular EAE, and our review will therefore mainly cover these preclinical studies.

2. Multiple sclerosis

According to current knowledge, an aberrant autoimmune response, mainly involving the adaptive immune system, causes MS. Vaccination of animals with myelin peptides induces different models of MS collectively known as EAE, characterized by different clinical courses (i.e. monophasic, relapsing-remitting, chronic). T lymphocytes are regarded as the principal effectors of MS, as shown by pathology studies showing perivascular CD4+ T cell infiltrates in brains of affected subjects, increased risk in those carrying specific HLA class II alleles, and by the fact that EAE can be mediated by transfer of myelin-reactive CD4+ T cells from affected to healthy animals (as reviewed in [46]). It has been hypothesized that autoreactive Th17 lymphocytes, primed in the periphery, initiate the disease by migrating into the CNS through the choroid plexus, while Th1 cells are involved in maintaining the immune responses in the CNS [47]. The first report on the use of MSCs to induce immune tolerance in experimental models of autoimmunity such as EAE came from our laboratory. Mice affected with progressive or relapsing EAE had an improved disease course after iv administration of syngeneic or allogeneic MSCs [48,49], which was associated to an impairment of myelin-specific and non-specific T-cell and B-cell responses. Such reports were confirmed by many subsequent studies that explored the impact of MSC treatment on EAE clinical and immunological features, and found that treatment with MSCs reduces the infiltration of immune cells in the CNS [50], and increases Th2 while decreasing Th1 and Th17 responses [51].

The possibility that MSCs could also impact the abnormalities in subsets of T cells with regulatory functions ($FOXP3^+$ regulatory T cells—Treg cells; T regulatory 1-Tr1 cells) that have been described in MS and likely contribute to the deregulated autoimmune response characterizing the disease (reviewed in [52]), was recently suggested by the study of Luz-Crawford et al. who showed that treatment with MSCs induces Foxp3 Treg cells in EAE [53].

B cells are also deeply involved in MS pathogenesis as demonstrated by the presence, in the cerebrospinal fluid of MS individuals, of oligoclonal immunoglobulin molecules produced by CNS-resident B cells (oligoclonal bands on electrophoresis) and of related B-cell clones from the brains of MS patients [54] (reviewed in [55]), as well as, more importantly, by the therapeutic effect of B-cell depletion in MS [56]. Decreased production of antibodies from ex vivo-isolated B cells was observed in mice with relapsing EAE treated with syngeneic MSCs [49]. Moreover, the treatment with MSCs was shown to induce CD1d(high)CD5(+) B regulatory cells in EAE [57].

In MS patients, particularly at the progressive phase, DCs display a pro-inflammatory profile suggesting that they could play a role in disease pathogenesis [58] (reviewed in [59]). It has been shown both in vitro and in vivo that MSCs affect DC activation and thereby their capacity to prime T cells [28,29].

The in vitro demonstration that MSCs can modulate microglia activation [30] suggested that MSC treatment could also affect microglia/macrophages in vivo. Activation of microglial cells has been demonstrated in EAE where it correlates with neuronal damage [60], and in MS, with possible increased incidence in progressive MS [61]. In MS brains, activated microglial cells and

macrophages are detected not only in MS lesions but also in normal-appearing white matter, with the development of "microglia nodules" which appear specific for MS pathology, are in association with degenerating axons and may develop in demyelinating lesions [62–64]. In EAE and MS, macrophages display a proinflammatory profile that may concur with disease worsening (reviewed in [59]). Recent animal studies suggest that microglia and monocytes may contribute differently to triggering EAE. A recent paper by Yamasaki et al. suggested that, at disease onset, macrophages derived from peripheral monocytes have a detrimental role and mediate demyelination, while microglial cells clear myelin debris and, differently from macrophages derived from periphery, are kept in a nonactivated state [65]. Fewer macrophages were detected in the spinal cord of mice treated with MSCs compared to those of control mice [48,49]. Bai et al. showed that factors secreted by MSCs, particularly hepatocyte growth factor (HGF), are sufficient to ameliorate disease in EAE; through blockade of its receptor cMet, which is expressed on both immune and neural lineage cells, including microglia, they demonstrated that the functional benefits of HGF in EAE were mediated by cMet [66]. While the effects of MSC-derived HGF on the adaptive immune cells in EAE are clear, biasing the T-cell response from Th1 to Th2, a possible direct effect of MSC-derived HGF administered iv on microglial cells is more speculative and dependent on the ability of HGF to cross or not the blood-brain barrier [66]. These results, together with the recurrent findings of an extremely limited CNS engraftment, not only following iv administration but also upon intracerebral administration [38], and the lack of significant transdifferentiation into neural cells [45,49,67] proved that MSC secretome, following iv injection, may suffice to recapitulate many of the effects carried out by these cells on immune and tissue resident cells including inhibition of CNS inflammation, neuroprotection, and induction of local neurogenesis.

All studies of MSCs in EAE cited above have employed bone marrow (BM)-derived MSCs but treatment with adipose-derived MSCs (ADMSC) has also been shown to be effective [68]. Payne and colleagues have tested the anti-inflammatory and therapeutic properties of green fluorescent protein (GFP)-labeled MSCs from different sources (human BM-derived MSCs, umbilical cord-derived MSCs and ADMSCs). Surprisingly, the authors found that BM-derived MSCs suppress the proliferation of splenocytes upon different stimuli to the maximum extent, but that ADMSCs were somewhat more effective in ameliorating the disease course of EAE; monitoring of the cells through PCR analysis of GFP gene expression in different tissues led the authors to hypothesize that this is mediated by increased migration of ADMSCs into the CNS compared to MSCs derived from other sources [69]. These results are in line with the observation that ADMSCs express activated α4 integrins and adhere to inflamed brain venules leading to a significant amelioration of EAE [68].

It is interesting to emphasize that syngeneic, allogeneic and xenogeneic (human) MSCs have been employed in the successful treatment of EAE and many other experimental conditions [10]. More importantly, allogeneic MSCs have been successfully administered in most human diseases. This is of significant relevance as MSCs, contrary to what is often considered, are not immune privileged, as they are quickly rejected by MHC class I- and class II-mismatched recipient mice. The immunological basis for this event lies in the ability of activated cells of the innate immunity such as NK and γδ T cells to kill allogeneic MSCs [70–71]. The interpretation of these findings fits with the idea that MSC effect is not based on engraftment and transdifferentiation into injured tissues but, most likely, on their ability to release, upon delivery, therapeutic molecules upon immediate interaction with the host environment [72]. Allogeneic MSC treatments, classified as a drug by regulatory agencies, have been widely used for therapeutic purposes in human diseases; however, the use of autologous BM-derived MSCs should be preferred in many conditions such as MS [73]. This is justified by the observation that, similarly to BM-derived MSCs from EAE mice that have a phenotype comparable to BM-derived MSCs from healthy syngeneic mice and identical clinical and anti-inflammatory effect [74], BM-derived MSCs from patients with MS are comparable to those from healthy subjects in terms of differentiation potential and expression of surface markers [75].

Some studies have explored the possibility to enhance the immunomodulatory effect of MSCs in EAE: similar effect on immune responses, but improved outcome of EAE have been observed with administration of MSCs transduced to express vasoactive intestinal peptide, a molecule with immunomodulatory and neuroprotective features [76]. Another strategy which employed ADMSCs overexpressing IL-10 had a higher impact on suppression of T-cell responses, with decreased proinflammatory cells in lymph nodes and decreased Th17 cells/Th1 cells ratio in the CNS as compared to unmodified MSCs, and resulted in a significantly greater amelioration of disease [77]. A recent study revealed that iv administration of MSCs with impaired autophagocytic capability through knockout of the expression of the autophagy-related *Becn1* gene was superior to the administration of control MSCs in ameliorating EAE. Based on in vitro data showing that inflammatory cytokines induce autophagy of MSCs, the authors suggest that the inflammatory environment of EAE may induce autophagic properties of MSCs that decrease their immunosuppressive function [78].

In summary, the treatment of EAE with MSCs is associated with clinical improvement that is itself related, beyond neuroprotection and neurorapair, to a modulation of peripheral pathogenic immune responses and inhibition of local inflammation mainly through the secretion of therapeutic molecules. Based on these findings an international consensus was generated [79], as the basis to design a large randomized, placebo-controlled clinical trial with autologous MSCs for the treatment of MS, which is currently ongoing: ancillary mechanistic studies will hopefully permit to assess the impact of MSC administration on immune responses in MS patients in relation with the clinical effects [73,79].

3. Alzheimer's disease

AD is a chronic neurodegenerative disease of the elderly characterized pathologically by extraneuronal deposits of Amyloid β protein (Aβ) (senile plaques) and intraneuronal tangles of tau protein, and clinically by progressive dementia with prominent memory loss. Pathology studies, experimental models and genetic studies reveal that AD is accompanied by an inflammatory response that is confined to the CNS, and recent studies suggest that the local inflammation, particularly aberrant behavior of microglial cells, possibly induced by the loss of local homeostasis, may contribute to AD pathogenesis [6]. In particular, mutations in the gene encoding the triggering receptor expressed on myeloid cells (TREM)-2 molecule expressed on macrophages/microglial cells have been associated to the risk of AD and other neurodegenerative diseases [80], although its role in the disease is controversial [81–82]. Induction of phagocytosis by stimulation of nuclear receptor agonists enhances Aβ clearance by both microglial cells and CNS macrophages ameliorating the disease course of experimental AD [83]. Other studies have hypothesized a role of adaptive immunity, and particularly Th1 cells, in the pathogenesis of AD ([84], reviewed in [85]).

MSC have been employed in treatment protocols of the experimental models of AD. Mice double transgenic for amyloid precursor protein and presenilin 1 (APP/PS1) present a progressive dementia associated with deposits of Aβ that resembles human AD.

Bilateral transplantation of human umbilical cord-derived MSC (hUCB-MSCs) increased the expression of enzymes degrading Aβ, such as neprilysin 1, by microglial cells and reduced the number of Aβ plaques in affected animals [86]. The authors of this study found that hUCB-MSCs released soluble intracellular adhesion molecule-1 (sICAM-1), which decreases Aβ plaques by inducing microglia to express neprilysin 1 via the sICAM-1/LFA-1 signaling pathway [86]. In the same animal model, repeated administration of hUCB-MSCs into the hippocampus bilaterally did not cause upregulation in the expression of Aβ degrading enzymes, such as neprysilin, by microglia; however, the authors showed that microglial cells had a more activated phenotype and a decreased expression of proinflammatory cytokines, like IL-1β and TNF-α, together with an increased expression of molecules associated to alternatively activated microglial phenotype, suggesting that the modulation of microglia may mediate the neuroprotective effect of MSC treatment [87]. Similarly, the same group demonstrated CCL5-mediated microglial recruitment and activation towards a beneficial phenotype after MSC transplantation [88]. Another study employed hUCB-MSCs that were pre-treated in vitro with tricyclodecan-9-yl-xanthogenate to induce a neuron-like phenotype. The so-called "neuralized" MSCs were then injected in the hippocampus of APP/PS1 double transgenic mice, with a beneficial effect on clinical parameters that was again associated with the induction of neuroprotective microglia; however, in contrast with the previous study, the authors did not see such effects when mice were treated with non-neuralized MSCs [89].

In conclusion, the innate immune system, and particularly microglial cells, participates to the pathogenesis of AD, although it is not clear whether the impairment in microglial functions has a causal effect or is the consequence of the abnormal CNS environment. Thus, the administration of MSC in AD might be beneficial mainly through changing microglia phenotype from classically to alternatively activated [30] resulting in their higher capability to degrade Aβ deposits. All single-target therapeutic approaches, mostly directed to Aβ, have so far failed and the multifactorial nature of the disease might be better addressed by MSC administration, which offers a multi-targeting therapeutic approach.

4. Amyotrophic lateral sclerosis

ALS is a lethal disease affecting the motor neurons and characterized by progressive weakness of skeletal muscles. Animal models and genetic forms of disease suggest that development of ALS is associated, among other mechanisms, to oxidative stress and abnormal protein degradation, which lead to damage of the motor neurons. An increased number of microglial cells with an activated status is classically described in brain and spinal cords of subjects deceased due to ALS (reviewed in [90]). A recent report by Endo et al. suggests that, in ALS, the phenotype of microglial cells is switched to a detrimental one by astrocytes, thus worsening disease outcome [91].

Mice with a glycine to alanine mutation at amino acid position 93 in the superoxide dismutase gene (SOD-G93A mice) are most commonly used to model human ALS, as they display features similar to human ALS in particular with regard to innate CNS responses. In this mouse model, Vercelli and colleagues showed that intraspinal cord injection of MSCs before the clinical onset of disease is associated with decreased microglial activation and decreased severity of murine ALS [92]. Similar effects of MSC administration on microglial cells were observed by Boucherie et al., who reported on transdifferentiation of MSCs into astrocytes after injection into the cerebrospinal fluid and hypothesized that the newly generated MSC-derived healthy astrocytes might affect the CNS microenvironment beneficially [93]. iv administration of allogeneic MSCs after the onset of motor symptoms prolonged the life span of SOD-G93A mice [94]. Injected MSCs scantly homed to the CNS, but a significant reduction of accumulation of ubiquitin agglomerates and of activated astrocytes and microglia was observed in the spinal cord of MSC-treated SOD1/G93A mice, together with a significant inhibition of the excessive release of glutamate [94]. Similarly, an impact on microglial phenotype with increased percentage of ramified, resting cells and a decreased number of activated cells upon intrathecal MSC treatment after disease onset was observed by Boido and coworkers [95].

While innate immunity appears detrimental in ALS, animal studies suggest that the adaptive immune system may play some beneficial role, as removal of T cells was associated to a worsened disease outcome (as reviewed in [85]). Treatment with MSCs was also shown to boost positive adaptive immune responses in ALS, leading to increased infiltration of immune cells into the spinal cord in the animal model [96]. In human beings with ALS or MS, concomitant intrathecal and iv treatment with autologous MSCs led to increased numbers of circulating Treg cells (assessed as CD4+ CD25+ T cells) [97]. Other findings of the latter study include a decreased number of circulating myeloid DC and decreased proliferation of lymphocytes in response to stimulus [97]. The authors conclude that these observations suggest a significant immunomodulatory effect of the treatment, although the clinical relevance of this effect could not be assessed.

In conclusion, an inflammatory signature characterizes innate immune cells in ALS. MSC treatment, by modulating the function of innate cells and possibly enhancing the beneficial effect of adaptive immunity, may support the possible use of MSCs in ALS.

5. Spinal cord injury

Neuronal death and breakdown of the blood brain barrier that occur in traumatic injury of CNS cause activation of local microglia and migration of monocytes from the periphery; such cells secrete proinflammatory molecules that aggravate the disease (reviewed in [98]). Migration of macrophages and microglial cells to injured tissues appears to have a biphasic pattern, with a first wave in the early phase post-trauma (1–13 days in the rat model) and a second wave at a later stage (14–180 days) [99]. While the first wave is known to be detrimental, reduced recruitment of macrophages in the later stage of disease is associated to poorer recovery, suggesting a beneficial role of innate late inflammatory response in SCI [99].

Yoshihara and colleagues treated rats with experimental SCI with MSCs, administered intraventricularly one hour after the lesion, and found decreased inflammatory responses (measured as levels of TNF-α) in the cerebrospinal fluid (CSF) three days after lesioning [100]. Nakajima et al. specifically investigated the impact of early (3 days) intralesional treatment with MSCs on macrophages in a rat model of SCI, finding that one week after the lesion, macrophages of treated rats acquired a beneficial, M2 phenotype, whereas macrophages from control rats had a detrimental, M1 phenotype [101]. Similarly, increased arginase activity, marker of alternative microglial/macrophage function, was observed in the spinal cord at day 7 post injury if rats had been treated with MSCs one day after the lesion [102]. Others found that iv injection of MSCs immediately after SCI was able to contrast the upregulation of pro-inflammatory cytokines IL-4 and TNF-α observed in untreated mice at 24 h, 72 h and 7 days post-injury [103]. Boido and coworkers injected MSCs intralesionally immediately after SCI and did not find differences in number and macroscopic appearance of microglial cells at a later stage (day 26 post lesion), despite improved clinical outcome: however the authors did not evaluate the phenotype of the cells (beneficial vs. detrimental) [104].

A reduced number of activated microglial/macrophages, as measured by count of CD68-positive cells, at six weeks post injury was found in spinal cord of rats treated with MSCs at day 7 post injury; similar results were obtained with MSCs genetically engineered to express multineurotrophin MNTS1, a neurotrophin, but the treatment was associated with improved axonal growth and sensory function, suggesting the possibility that MNTS1 shuttled by MSCs can improve neural repair [105].

MSCs genetically engineered to overexpress cerebral dopamine neurotrophic factor, and transplanted at the lesion site immediately after lesion, were more effective than treatment with control MSCs in reducing local inflammation after SCI in rats, with reduced expression of prostaglandin E2, IL-1β and TNF-α [106]. The impact of the timing of intralesional MSC transplantation (12 h, 1 week, 2 weeks after injury) on inflammation (measured as expression of cyclooxygenase-2 - COX-2) and functional outcome was assessed in the dog model of SCI; a decrease in COX-2 was observed in both the 1- and 2-week treatment groups as compared to the 12-h transplantation group, whereas there were no significant differences at the 12-h time point, and the authors suggest that early MSC treatment in SCI may be impeded at the peak inflammation period [107].

Finally, treatment with MSCs was shown to have a beneficial impact on neuropathic pain after SCI though modulation of microglial function and decreased recruitment of peripheral macrophages into the spinal cord [108]. In conclusion, treatment with MSCs may have a positive effect in SCI through dampening of the detrimental inflammation and strengthening of the positive inflammatory response, which is associated to some repair.

6. Ischemic stroke

Ischemic stroke is characterized by the permanent or transient occlusion of a blood vessel due to a thrombus or embolus, which, when not lethal, often results in permanent neurological impairment due to progressive neuronal cell death through necrosis or apoptosis associated with subsequent neuroinflammation. Since 2000, several reports have reported a remarkable neuroprotective effect of MSC-based therapies in rodent stroke models. The model most used for pre-clinical studies is that induced by middle cerebral artery occlusion (MCAO). In a seminal study, Li et al. described transplantation of non-hematopoietic cells from adult mouse bone marrow into the striatum four days after embolic MCAO and observed that functional recovery was significantly improved in transplanted mice monitored for 28 days as compared with untreated mice [109]. In 2001, Chen et al. demonstrated that 5-bromo-2′-deoxyuridine (BrdU)-labeled rat MSCs, injected iv into rats one or seven days after stroke induction, improved neurological functional deficits [110]. In a subsequent study, the same group tested the effect of human MSCs (hMSCs) injected iv one day after stroke induction in rats, and showed that only a few (1–5%) hMSCs could be detected in the CNS; this was associated with a decrease in apoptotic cells in the ischemic boundary zone accompanied by an increase in brain-derived neurotrophic factor and nerve growth factor in cerebral tissue, as well as an increase in proliferation of endogenous cells in the subventricular zone [111]. More recently, Ishika et al. demonstrated that also following intra-arterial hMSC transplantation one or four days after MCAO, functional recovery can be successfully achieved; however, only a few cells could be detected early in the core and in the peri-infarct area, associated with a significant decrease of microglia activation, increased number of reactive astrocytes, and enhanced angiogenesis [112]. Similar data were confirmed by many others suggesting that even following intra-arterial administration, MSCs poorly engraft in the brain following cerebral ischemia despite a significant functional recovery [113].

Promotion of angiogenesis following iv infusion of hMSCs early after stroke induction was also demonstrated by Chen and colleagues together with an increased secretion of vascular endothelial growth factor thereby resulting in improved neurological recovery [114]. Subsequent studies have attempted to improve the neuroprotective effect of MSCs through genetic modification. Liu et al. showed that both hMSCs or hMSCs transfected with angiogenic placental growth factor (PlGF) gene (PlGF-hMSCs) injected iv into rats three hours after MCAO reduced lesion volume, induced angiogenesis, and elicited functional improvement compared with the control group, but the effect was greater in PlGF-hMSC-treated rats [115]. In a recent study, Xin et al. observed that iv treatment of rats with MSCs after MCAO significantly increased neuronal levels of miR-133b, a miRNA they showed to be transferred from MSCs to neurons and astrocytes, resulting in a significant increase in neurite branch numbers and total neurite length [116–117]. Interestingly, stroke induces changes in the miRNA profile of MSCs and within their released exosomes [118]. In line with the broad neuroprotective effect exerted by MSCs, Van Velthoven et al. demonstrated that local administration of MSCs could restore functional cortical rewiring and increase axonal connectivity in the ipsilesional hemisphere [119]. Remarkably, also intranasally administered MSCs can decrease brain damage upon neonatal stroke [120]. In a model of stroke in rodents, it was recently demonstrated that transplantation of TGF-β-secreting MSCs at early post-ischemia inhibited the upregulation of MCP-1/CCL2 in the ischemic area resulting in a reduced infiltration of CD68-positive immune cells through the blood brain barrier (BBB) [121]. These data are consistent with a recent report showing that MSC therapy reduced ischemia-induced aquaporin-4 upregulation by astrocytes via p38 signaling pathway promoting BBB integrity due to inhibition of inflammation [122]. Accordingly, MSC infusion was recently associated with a significant inhibition of the expression of IL-23 and IL-17 around infarct lesion in mice after stroke [123]. Scheibe et al. raised the question of possible deleterious effects of MSCs on the immune response in stroke in view of the stroke-induced immunodepression associated with cerebral ischemia, which predisposes to bacterial infections with increased mortality. Despite their immunosuppressive effects in vitro, transplantation of MSCs in mice after MCAO did not affect the serum levels of relevant inflammatory cytokines, suggesting that safety concerns for MSC transplantation in stroke are likely to be unwarranted [124].

In conclusion the therapeutic effect of MSCs in stroke may not only be based on neuroprotection but may occur also through an anti-inflammatory effect and, more importantly, through the induction of angiogenesis and restoration of BBB integrity.

7. Conclusion

Immune responses occurring in the CNS and in periphery have a significant impact on the course of chronic neurological diseases: adaptive and innate immunity in MS, and mostly innate immunity in neurodegenerative diseases, are not just observers but contribute, in ways that are complex and still poorly known, to the neuronal damage associated to different forms of disability. The effects of MSCs on adaptive and innate immune responses appear to mediate, at least in part, their therapeutic efficacy in CNS diseases. The acquisition of new insights on the immunopathogenesis of CNS diseases and on the interactions between immune cells, mediators of inflammation, and neural cells should be taken into account by future studies evaluating the effect of MSCs on neurological diseases. MSCs, as a double-barrel weapon in CNS diseases that provide, at the same time, neuroprotection and immunomodulation, represent a fascinating approach to the prevention of disability. Current clinical trials should answer fundamental ques-

tions regarding, not only safety, but more importantly, efficacy and mode of action of MSCs in specific neurological diseases, with particular focus on immune responses that are associated to the development of irreversible disability.

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