PERSPECTIVE

Mesenchymal stem cells require the peripheral immune system for immunomodulating effects in animal models of multiple sclerosis

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) that affects oligodendrocytes and myelin. Loss of myelin leads to progressive axonal damage and neuronal death resulting in neurodenegeration and functional disability. Several inflammatory factors influence the development of this neurological disorder. It is generally accepted that autoreactive T lymphocytes migrate towards the CNS and then initiate an immune reaction upon encountering the specific myelin antigen (Mahad et al., 2015). Remyelination is the natural repair mechanism and is important to restore the fast saltatory nerve conduction. In addition, it restores the axon-myelin unit and may thus preserve the axon from secondary degeneration. This regenerative process implies the migration of oligodendroglial precursor cells (OPC) towards demyelinated regions and their differentiation into mature myelinating oligodendrocytes (Franklin, 2015). Unfortunately, this process is often incomplete in MS patients and current treatments are based on the use of immunomodulatory drugs, which diminish the inflammatory reaction, but they do not repair existing damage. For this reason, extensive research is being conducted in the area of remyelinating therapies in order to stop disease progression and restore neurological disabilities.

A useful tool to investigate de- and remyelination processes is the use of *in vivo* animal models that represent different aspects of the complex pathophysiology of MS. The most studied animal model of MS is experimental autoimmune encephalomyelitis (EAE). In active EAE animals are immunized with purified myelin or myelin proteins such as myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG), or myelin associated glycoprotein (MAG). Consequently, reactive myelin specific T lymphocytes infiltrate the brain and spinal cord parenchyma and lead to immune mediated myelin destruction. Passive EAE is induced by transfer of activated myelin specific T cells. Similarly, these T lymphocytes generate an immune reaction that leads to demyelination (Robinson et al., 2014). This animal model resembles some aspects of human MS, but de- and remyelination processes overlap in time which complicates evaluation of remyelination. Furthermore, in this model the blood-brain barrier is disrupted. For this reason, infiltration of peripheral immune cells and soluble molecules into the brain complicate the analysis of CNS derived factors responsible for influencing remyelination (Al Jumah and Abumaree, 2012). To study remyelination, toxic models such as the lysolecithin or the cuprizone model are preferred. Cuprizone is a copper chelator, which selectively affects mature oligodendrocytes in the CNS. The feeding of cuprizone leads to nearly complete loss of oligodendrocytes after 3 to 4 weeks of treatment. Activation of astrocytes and microglia occurs and nearly complete demyelination of the corpus callosum is visible after 5 weeks of treatment. At this time point demyelination can be studied or animals can return to normal food chow to allow remyelination. Although the induction of demyelination is artificial the advantage is that both de- and remyelination processes can be studied independently without the influence of the peripheral immune system (Skripuletz et al., 2011). Hence, the use of this model can be helpful to determine the mechanisms of action of remyelinating therapies.

Among possible remyelinating approaches, mesenchymal stem cells (MSC) are in the focus of many studies due to their immunopriviledged state and immunoregulatory and neuroregenerative properties (Uccelli et al., 2008). Their beneficial effect has been widely investigated in models of neuroinflammation. Recently, an improvement in the EAE model was observed with enhancement of regeneration when MSC were applied intravenously or directly into the lateral ventricle (Zappia et al., 2005; Kassis et al., 2008). Although the mechanisms of action were not fully understood, the beneficial effects were suggested to be mediated by a direct suppression of the ongoing immune reaction. T lymphocytes could be inactivated through cellular interactions and paracrine factors such as prostaglandin E2, tumor growth factor β (TGFβ), hepatocyte growth factor (HGF), human leukocyte antigen G isoform, indoleamine 2,3-dioxygenase (IDO), interleukin 10, and metalloproteinases. The combination of these factors might block T cell proliferation and thus avoid their migration towards the CNS. Additionally, MSC might reduce the ongoing inflammation by downregulating the production of interferon gamma (IFNγ), interleukin 2, and tumor necrosis factor α (TNFα). However, it is not known if stem cells require the activation of the peripheral immune system for its supposed beneficial effect in regeneration processes. Recently, the cuprizone model was employed to analyze the effects of MSC on remyelination. As mentioned above, in this model remyelination is not influenced by the peripheral immune system. In our lab, intravenous and intranasal administration of MSC in mice during ongoing demyelination did not show any beneficial effects in comparison with untreated cuprizone controls. However, most of the intravenously transplanted MSC became trapped in the lungs and neither these nor the intranasally injected MSC reached the CNS, presumably because the blood-brain barrier remains intact in this model (Nessler et al., 2013). In the next step, MSC were injected intraventricularly and directly into the corpus callosum (Salinas Tejedor et al., 2015). Again, remyelination was not influenced by MSC in this toxic model. Thus, our current and previous results suggest that the presence of the peripheral immune system is required to achieve MSC-mediated regenerative effects in the CNS (**Figure 1**). In EAE effector and memory T cells reside within the bronchus-associated lymphoid tissues of the lungs and lung-draining mediastinal lymph nodes, where they are primed before infiltrating the CNS (Odoardi et al., 2012). While MSC become mostly trapped in the lungs after intravenous application (Nessler et al., 2013) we speculate that this effect might take place in the lungs independent of the mechanism of action of MSC (direct cellular contact and/or paracrine effects). In the lungs MSC might induce T cell anergy resulting in prevention of further damage. Bone marrow MSC-derived neural progenitor cells (MSC-NP) present an alternative source of stem cells. MSC-NP represent a subpopulation of bone marrow MSC with neural progenitor and immunoregulatory characteristics. Treatment with MSC-NP starting at the onset of the chronic EAE phase reduced disease symptoms, the inflammatory CNS reactions, and the area of demyelination (Harris et al., 2012). In addition, higher numbers of endogenous nestin positive progenitor cells were observed suggesting beneficial effects on repair processes.

MSC possess an immunopriviledged state because they lack HLA class II antigens and T cell costimulatory molecules (Uccelli et al., 2008). For this reason, the immune system of the host does not recognize MSC after transplantation and therefore it does not start an immune reaction against them. Thus, use of immunosuppressive drugs is not required in clinical trials. Currently, clinical trials with stem cells have been already conducted in several human disorders such as ischemic stroke, Crohn's disease, cardiomyopathy, myocardial infarction, graft *versus*

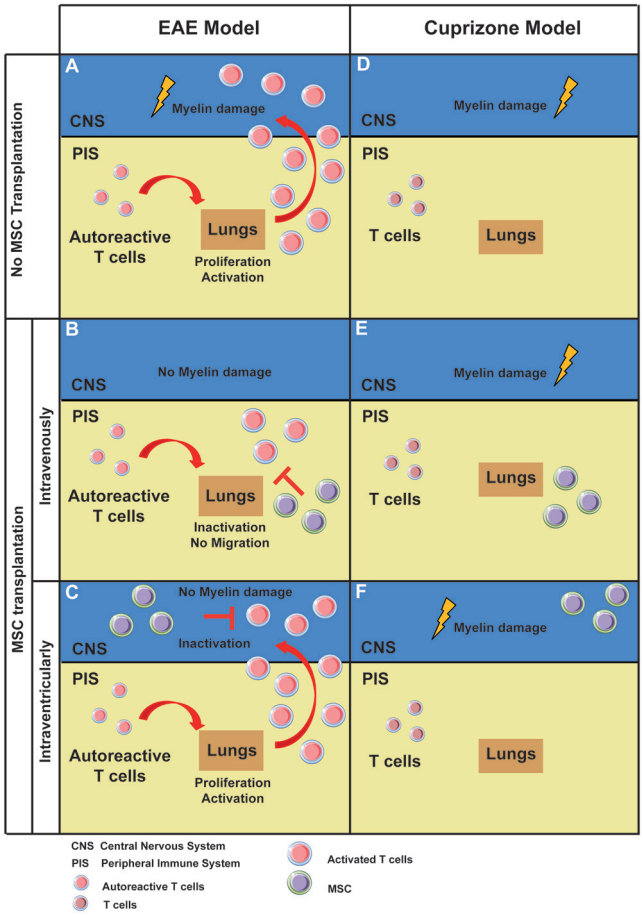

Figure 1 Schematic representation of the proposed mechanism of action of mesenchymal stem cells (MSC) in the experimental autoimmune encephalomyelitis (EAE) and the cuprizone model.
In EAE, autoreactive T cells are primed in the lungs where they become activated and proliferate before infiltration of the central nervous system. (A) After intravenous injection, MSC become trapped in the lungs and exert their immuregulatory properties inducing T cell anergy. (B) After intraventricular injection, MSC encounter T cells in the brain and the immune reaction is suppressed. (C) In contrast, in the cuprizone model where demyelination is not triggered by the peripheral immune system and T cells are not present in the lesions, (D) MSC injected intraventricularly or intravenously do not prevent from further myelin damage (E, F).

host disease, and amyotrophic lateral sclerosis. Furthermore, several clinical trials in phase I/II have been conducted to assess the safety of MSC therapy in patients with relapsing-remitting and progressive forms of MS. Allogenic MSC transplantation involved healthy donors. Other studies included injections of autologous MSC derived from the bone marrow of MS patients or autologous bone marrow derived MSC-NP. The mostly used administration routes applied in these clinical trials were intravenous or intrathecal applications. The side effects that followed intravenous injection were type I hypersensitivity. Side effects after intrathecal application included transient headache and fever. In general no significant side effects were observed to date (Dulamea, 2015).

In conclusion, MSC might present an interesting option in modulating the immune system in neuroinflammatory diseases such as MS. However, numerous questions are not answered to date such as the amount of cells and the frequency of treatment necessary for effectiveness as well as the risk of long term side effects such as development of tumors. Thus, further studies are needed including animal experiments and extrapolation from animal models to a possible human therapy that ultimately requires further investigation in large clinical trials.

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