Brain, Behavior, and Immunity xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Mesenchymal stem cells do not exert direct beneficial effects on CNS remyelination in the absence of the peripheral immune system

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ARTICLE INFO

Article history:
Received 30 March 2015
Received in revised form 17 June 2015
Accepted 29 June 2015
Available online xxxx

Keywords:
MSC
MS
Cuprizone
Intraventricular
Corpus callosum

ABSTRACT

Remyelination is the natural repair mechanism in demyelinating disorders such as multiple sclerosis (MS) and it was proposed that it might protect from axonal loss. For unknown reasons, remyelination is often incomplete or fails in MS lesions and therapeutic treatments to enhance remyelination are not available. Recently, the transplantation of exogenous mesenchymal stem cells (MSC) has emerged as a promising tool to enhance repair processes. This included the animal model experimental autoimmune encephalomyelitis (EAE), a commonly used model for the autoimmune mechanisms of MS. However, in EAE it is not clear if the beneficial effect of MSC derives from a direct influence on brain resident cells or if this is an indirect phenomenon via modulation of the peripheral immune system. The aim of this study was to determine potential regenerative functions of MSC in the toxic cuprizone model of demyelination that allows studying direct effects on de- and remyelination without the influence of the peripheral immune system. MSC from three different species (human, murine, canine) were transplanted either intraventricularly into the cerebrospinal fluid or directly into the lesion of the corpus callosum at two time points: at the onset of oligodendrocyte progenitor cell (OPC) proliferation or the peak of OPC proliferation during cuprizone induced demyelination. Our results show that MSC did not exert any regenerative effects after cuprizone induced demyelination and oligodendrocyte loss. During remyelination, MSC did not influence the dynamics of OPC proliferation and myelin formation. In conclusion, MSC did not exert direct regenerative functions in a mouse model where peripheral immune cells and especially T lymphocytes do not play a role. We thus suggest that the peripheral immune system is required for MSC to exert their effects and this is independent from a direct influence of the central nervous system.

1. Introduction

Multiple sclerosis is an inflammatory demyelinating disease of the central nervous system (CNS) that leads to progressive axonal damage and consequently to a loss of neurological functions (Lassmann, 2014). Remyelination is the natural mechanism of repair and it was supposed that it might protect from axonal loss and neurodegeneration (Tanaka and Yoshida, 2014). However, due to unknown reasons, remyelination is often incomplete or fails in MS patients (Bhatt et al., 2014). Currently licensed treatments for MS patients are limited to immunomodulatory drugs which are not able to directly enhance remyelination. Recently, the transplantation of exogenous mesenchymal stem cells (MSC) has been proposed to have such regenerative functions (Patel and Genovese, 2011).

MSC treatment has already been reported to be beneficial in the experimental autoimmune encephalomyelitis (EAE) model of MS (Morando et al., 2012). MSC are multipotent cells that can differentiate into adipocytes, chondrocytes, osteoblasts, and they have been observed to transdifferentiate into other cell types such as neural-like cells *in vitro* (Tondreau et al., 2008). Nevertheless, some controversy exists about the effect that MSC may have in CNS lesions and whether the homing of MSC to injured tissues and their engraftment is strictly required for their beneficial effect (Morando

Abbreviation: RFP, red fluorescent protein.

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http://dx.doi.org/10.1016/j.bbi.2015.06.024
0889-1591/

et al., 2012; Uccelli and Prockop, 2010). The main effects of MSC are suggested to be mediated by creating a neuroprotective and regenerative environment since MSC are able to secrete growth factors, matrix metalloproteinases, and chemokines such as transforming growth factor-beta (TGFβ), prostaglandin E2, hepatocyte growth factor, and interleukin 10, which could modulate both innate and adaptive immune responses (Uccelli et al., 2008; Rafei et al., 2009; Chen et al., 2011).

Here we investigated the direct effect of exogenously applied MSC on remyelination in the cuprizone model of toxic induced demyelination. The cuprizone model is widely accepted to study remyelination in the CNS (Gudi et al., 2014; Kipp et al., 2009; Skripuletz et al., 2011a). The aim of our experiments was to use an animal model of remyelination in which the peripheral immune system does not play an important role (Skripuletz et al., 2011a). This allows us to analyze the pathomechanisms of remyelination directly in the CNS bypassing interferences of peripheral immune cells, which might be found in inflammatory models such as EAE. EAE is a widely accepted animal model to study CNS inflammation and neurodegeneration, but assessment of remyelination may be complicated in a setting with concomitant de- and remyelination (Tanaka and Yoshida, 2014). To study remyelination, we have used different treatment protocols and MSC from three species (human, murine, canine) that were transplanted either intraventricularly into the cerebrospinal fluid or directly into the lesion of the corpus callosum.

2. Materials and methods

2.1. Experimental design

Demyelination was induced by feeding ten-week old mice a diet containing 0.2% cuprizone (biscyclohexanone oxaldihydrazone, Sigma–Aldrich) for 5 weeks *ad libitum*. After this period, animals returned to normal diet for remyelination (Gudi et al., 2014).

To analyze the effects of MSC treatments, different injection protocols were applied including 16 groups of mice (for experimental design, see Fig. 1). First, MSC from three different species were injected in separate groups: human MSC, murine MSC, canine MSC, and PBS sham injections for controls. Second, two administration pathways were chosen and MSC or sham in controls were applied intraventricularly into the right ventricle or intralesionally into the corpus callosum. Third, injections were performed at two different time points: week 3 which corresponds to a time point of onset of oligodendrocyte progenitor cell (OPC) proliferation and microglial recruitment in the cuprizone model or week 4 which corresponds to the peak of OPC proliferation and microglial activation (Skripuletz et al., 2011b). A group size of six mice was analyzed at the time point of complete demyelination in the corpus callosum (week 5) and the time point of early remyelination (week 5.5).

In order to exclude the influence of a stereotactic injection in the cuprizone model mice received an intraventricular PBS injection at week 4, which corresponds to the peak of OPC proliferation and microglial activation (Skripuletz et al., 2011b). Mice were analyzed at the time point of complete demyelination in the corpus callosum (week 5) and at the time point of early remyelination (week 5.5) and compared to control animals that were fed with cuprizone but did not receive an intraventricular injection. A group size of six mice was analyzed at both groups and both time points.

2.2. Animals

Male *C57BL/6* mice were purchased from Charles River (Sulzfeld, Germany). Animals were housed two per cage and rested one week for adaptation to the housing conditions prior use. All procedures involving animals were performed in compliance with the international guidelines on animal care in experimentation and approved by the Review Board for the Care of Animal Subjects of the district government (Lower Saxony, Germany).

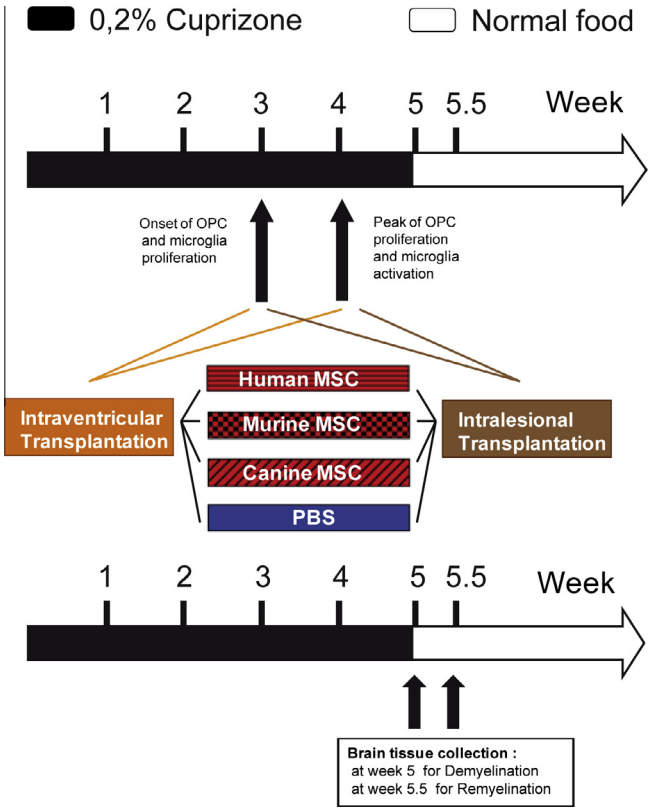

Fig. 1. Experimental design. Mice were fed with cuprizone for 5 weeks to induce demyelination. After week 5 mice were fed with normal food to allow remyelination. MSC from three different species (human, murine, canine) or PBS, as control, was transplanted either into the lateral ventricle or into the lesion of the corpus callosum. Cells or PBS were injected at two different time points: week 3 which corresponds to the onset of OPC and microglia proliferation, or week 4 which corresponds to the peak of OPC proliferation and microglial activation. Brains were collected and studied at the peak of demyelination (week 5) or at early remyelination (week 5.5).

2.3. Culture of MSC

Human MSC were isolated from bone marrow of a healthy donor as previously described (Nessler et al., 2013; Schack et al., 2013). Human MSC were seeded in a 175 cm^2 culture flask and incubated at 37 °C, 5% CO_2, 85% humidity. After 24 h, non-adherent cells were removed by washing with PBS. Additional medium changes were performed every 3 days. Before reaching confluence, MSC were harvested with trypsin–EDTA (Biochrom) and trypsinization was stopped with medium containing 10% fetal calf serum. Afterwards, subcultures were prepared at a density of 4000 cells/cm^2. MSC from passages 6 to 8 were characterized by flow cytometric analysis (FACS) and used for transplantation.

Murine MSC derived from bone marrow of C57BL/6 mice expressing red fluorescent protein (RFP) were purchased from Cyagen Cells and were cultured and expanded in T75 flasks with OriCell™ mouse MSC growth medium (Cyagen). Medium was changed every 2–3 days. Once 80–90% confluence was reached, cells were passaged by rinsing the cell surface 2 times with PBS without Ca^{2+} and Mg^{2+} (Gibco) and by incubating them with trypsin–EDTA for 5 min, at 37 °C. Cells between passages 11–13 were used for FACS analysis and transplantation.

Please cite this article in press as: Salinas Tejedor, L., et al. Mesenchymal stem cells do not exert direct beneficial effects on CNS remyelination in the absence of the peripheral immune system. Brain Behav. Immun. (2015), http://dx.doi.org/10.1016/j.bbi.2015.06.024