BRAIN RESEARCH 1452 (2012) 173–184

Available online at www.sciencedirect.com

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BRAIN RESEARCH

Research Report

Bone marrow mononuclear cells and mannose receptor expression in focal cortical ischemia

Arthur Giraldi-Guimarães[a,b], *Helder Teixeira de Freitas*[a], *Bárbara de Paula Coelho*[a], *Hugo Macedo-Ramos*[c], *Rosalia Mendez-Otero*[b], *Leny A. Cavalcante*[c], *Wagner Baetas-da-Cruz*[c,d,*]

[a]*Laboratório de Biologia Celular e Tecidual, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro—UENF, Campos dos Goytacazes, RJ, CEP: 28013-602, Brazil*

[b]*Laboratório de Neurobiologia Celular e Molecular, Programa de Terapia Celular e Bioengenharia, Instituto de Biofísica Carlos Chagas Filho, CCS, Universidade Federal do Rio de Janeiro—UFRJ, Rio de Janeiro, RJ, CEP: 21941-902, Brazil*

[c]*Laboratório de Neurobiologia Comparativa e do Desenvolvimento, Programa de Terapia Celular e Bioengenharia, Instituto de Biofísica Carlos Chagas Filho, CCS, Universidade Federal do Rio de Janeiro—UFRJ, Rio de Janeiro, RJ, CEP: 21941-902, Brazil*

[d]*Universidade Federal do Rio de Janeiro—UFRJ, Campus Macaé, Cidade Universitária, Macaé, RJ, CEP: 27930-560, Brazil*

ARTICLE INFO

Article history:
Accepted 1 March 2012
Available online 8 March 2012

Keywords:
Stroke
Cell therapy
Stem cell
Repair
Pattern recognition receptors (PRRs)
Damage-associated molecular patterns (DAMPs)

ABSTRACT

The use of bone marrow mononuclear cells (BMMCs) has been shown as a putative efficient therapy for stroke. However, the mechanisms of therapeutic action are not yet completely known. Mannose receptor (MR) is a subgroup of the C-type lectin receptor superfamily involved in innate immune response in several tissues. Although known primarily for its immune function, MR also has important roles in cell migration, cell debris clearance and tissue remodeling during inflammation and wound healing. Here we analyzed MR expression in brains of rats one week after induction of unilateral focal cortical ischemia by thermocoagulation in blood vessels of sensorimotor cortex. Additionally, we evaluated possible changes in such expression in cortices of rats subjected to ischemia plus treatment with BMMCs. Our results showed high expression of MR in an unknown $GFAP^+$ cell type and in phagocytic macrophages/microglia within the lesion boundary zone whereas in the non-injured (contralateral) cortical parenchyma, low levels of MR expression were observed. Moreover, therapy with BMMCs induced overexpression of MR in ipsilateral (injured) cortex. Previous studies from our group have shown functional recovery and decreased neurodegeneration in BMMC-treated rats in the same model of focal cortical ischemia. Thus, we suggest that ischemic injury induces large increase in MR expression as part of a mechanism for clearance of damage-associated molecular patterns (DAMPs). In addition,

* *Corresponding author at:* Center of Experimental Surgery, Department of Surgery, Medical Faculty, Federal University of Rio de Janeiro, Av. Carlos Chagas Filho 373, CCS, Bloco J, 2°and, 21941-902, Rio de Janeiro, Brazil. Fax: +55 21 2280 8193.
E-mail addresses: wagner.baetas@ufrj.br, wagner.baetas@pq.cnpq.br (W. Baetas-da-Cruz).

Abbreviations: ANOVA, analysis of variance; BMMCs, bone marrow mononuclear cells; Man-BSA, mannosylated bovine serum albumin; DAMPs, damage-associated molecular patterns; DAPI, 4′,6-diamidino-2-phenylindole; ECL, enhanced chemiluminescence; FITC, fluorescein isothiocyanate; IB4, isolectin B4; IHC, immunohistochemistry; HRP, horseradish peroxidase; MR, mannose receptor; OD, optical density; PBS, phosphate-buffered saline; PRRs, pattern recognition receptors; SEM, standard error mean

doi:10.1016/j.brainres.2012.03.002

induction of MR overexpression by BMMCs might increase the efficiency of clearance, being one of the protective mechanisms of these cells.

1. Introduction

Systemic administration of bone marrow-derived cells is a promising strategy to treat stroke. Beneficial effects of these cells have been described in animal models of ischemic stroke (Brenneman et al., 2010; Chopp and Li, 2002; de Vasconcelos dos Santos et al., 2010; Giraldi-Guimarães et al., 2009; Iihoshi et al., 2004; Kamiya et al., 2008; Mezey, 2007). Moreover, the feasibility and safety of therapy for stroke in humans with autologous bone marrow cells have already been demonstrated in clinical studies (Bang et al., 2005; Barbosa da Fonseca et al., 2010; Battistella et al., 2011; Savitz et al., 2011).

Transplantation of bone marrow mononuclear cells (BMMCs) has been shown to be an effective protocol for cell therapy, since BMMCs can be obtained without cultivation and can be harvested in 1.5–6 h for autologous administration (Battistella et al., 2011; Brenneman et al., 2010; Iihoshi et al., 2004; Savitz et al., 2011). Thus, treatment can be performed during acute phase of stroke (Mendez-Otero et al., 2007). BMMCs are a heterogeneous cell fraction composed by hematopoietic stem, progenitor and differentiated cells, mesenchymal stem cells and endothelial progenitor cells (Orkin, 2000; Pittenger et al., 1999; Wang et al., 2008; Weissman et al., 2001). This cell composition gives to BMMCs important therapeutic features, such as multipotentiality, chemoattractiveness to lesioned tissues and capacity to produce and release many cytokines and trophic factors (Chopp and Li, 2002; Crain et al., 2005; Kawamoto and Losordo, 2008; Krause et al., 2001; Shyu et al., 2006). In fact, BMMC mechanisms of protection in brain ischemia have been shown to involve mainly chemoattraction to lesion and local paracrine action, by release of many anti-inflammatory, angiogenic and neuroprotective factors (Mendez-Otero et al., 2007). However, the whole mechanism is currently being discovered and all restorative processes triggered by BMMC paracrine action are still not completely known.

Here we investigated a possible effect of BMMC treatment in tissue clearance in cerebral ischemia. For this reason, we analyzed the expression of mannose receptor (MR), an important player in this process. Mannose receptor (MR) is a subgroup of the C-type lectin receptor superfamily involved in innate immune response in several tissues, including nervous tissue (Linehan et al., 1999). MR is a constitutive receptor, which under influence of immune mediators acts as a pivotal molecule in the host defense mechanism, being a link between innate and adaptive immunity (Areschoug and Gordon, 2008). Although MR is known primarily for its innate immune function, it also has an important role in cell migration, intracellular signaling, cell debris clearance and tissue remodeling during inflammation and wound healing (Gazi and Martinez-Pomares, 2009; Taylor et al., 2005).

Thus, we analyzed MR expression in the cerebral cortex of rats that underwent focal ischemia induced by thermocoagulation of blood vessels in primary motor and somatosensory cortices. Additionally, we evaluated possible changes in cortical pattern of MR expression in rats subjected to cortical ischemia followed by treatment with BMMCs. We chose the time window of a week after ischemia induction for our analyses. This post-ischemia time is likely close to the end of the acute phase, since the cortical tissue is almost degenerated, but not completely (Szele et al., 1995), and inflammatory events and clearance are still occurring. Moreover, recent reports have suggested that systemically injected BMMCs migrate to an injured cortex as fast as 1 h after injection, but they undergo progressive death, being almost absent in an ischemic lesion 7 days after injection (Brenneman et al., 2010). We repeated the previously used protocol of treatment 24 h after ischemia induction (Giraldi-Guimarães et al., 2009). Our rationale was to perform analysis in a time point still inside the acute phase, but with as few as possible exogenous BMMCs. Since the mononuclear fraction contains monocytes with potential to differentiate in MR-expressing macrophages, their presence in earlier time points could mask the intensity of MR expression by endogenous cells within the ischemic lesion.

2. Results

In accordance with previous descriptions (de Vasconcelos dos Santos et al., 2010; Giraldi-Guimarães et al., 2009), our procedure of thermocoagulation induced a consistent ischemic lesion that included the six cortical layers, sparing white matter (Fig. 1). The time window after ischemia induction

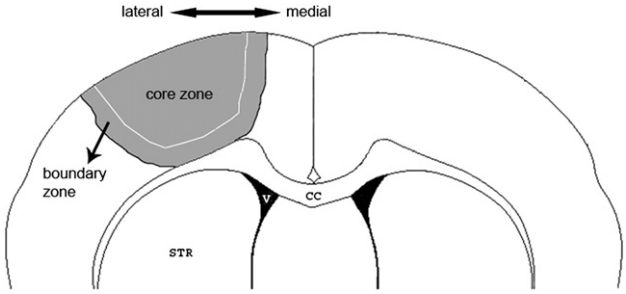

Fig. 1 – Lesion regions. Illustration showing ischemic lesion extension observed in our experiment, with two regions identifiable after seven post-ischemic days. White line indicates the limit between core and boundary zones, and black line indicates the cortical limit of the lesion (represented in gray). The core zone is characterized by greatly altered cytoarchitecture, often with large spaces formed by tissue cleaning (not shown). The boundary zone presents more preserved cell organization, favoring a better cytological analysis. The lateral–medial axis is indicated for the left hemisphere. STR = striatum; V = lateral ventricle; CC = corpus callosus.