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Research Report

Nitric oxide-mediated immunosuppressive effect of human amniotic membrane-derived mesenchymal stem cells on the viability and migration of microglia

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ARTICLE INFO

Article history:
Accepted 26 May 2014
Available online 6 June 2014

Keywords:
Stem cell
Immunology
Cell therapy

ABSTRACT

Human amniotic membrane-derived mesenchymal stem cells (AMSCs) are considered a novel and promising source of stem cells for cell replacement-based therapy. Current research is mostly limited to investigating the cellular differentiation potential of AMSCs, while few have focused on their immunosuppressive properties. This study is aimed at exploring and evaluating the immunosuppressive effect of human AMSCs on the viability and migratory properties of microglia. We found, from results of cell viability assays, that AMSCs can reduce the activity of inflammatory cells by secreting nitric oxide (NO). Also, based on results from wound healing and transwell migration assays, we show that AMSCs can inhibit the migration of human microglia as well as the mouse microglial cell line BV2, suggesting that they have the ability to inhibit the recruitment of certain immune cells to injury sites. Furthermore, we found that NO contributes significantly to this inhibitory

Abbreviations: AMSCs, amniotic membrane-derived mesenchymal stem cells; NO, nitric oxide; CNS, central nervous system; MSCs, mesenchymal stem cells; MHC, major histocompatibility complex; HLA, human leukocyte antigen; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; CM, conditioned medium; AMSC-CM, AMSCs conditioned medium; NOS, nitric oxide synthase; SMT, S-methylisothiourea sulfate; PGE2, prostaglandin E2; TGF-β1, transforming growth factor beta 1; STAT5, signal transducer and activator of transcription 5; PBS, phosphate-buffered saline; DMEM/F-12, Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12; FBS, fetal bovine serum; CCK-8, Cell Counting Kit-8; OD, optical density

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http://dx.doi.org/10.1016/j.brainres.2014.05.041

effect. Our study provides evidence that human AMSCs can have detrimental effects on the viability and migration of microglia, through secretion of NO. This mechanism may contribute to anti-inflammatory processes in the central nervous system.

1. Introduction

Mesenchymal stem cells (MSCs) are a promising resource for cell replacement therapy-based clinical applications. They can be isolated from many adult tissues, including bone marrow, adipose tissue, placenta, amnion and cord blood (Shi et al., 2012). However, compared to MSCs derived from adult sources, AMSCs have more useful properties owing to their derivation from an early embryological stage. Their differentiation potential is less restricted, and they display low levels of expression of major histocompatibility complex (MHC) antigens (Miki and Strom, 2006; Manuelpillai et al., 2011). Several studies have reported that AMSCs can differentiate into neurons, cardiomyocytes, alveolar epithelium and pancreatic b-islet cells after transplantation, and can secrete proteins normally produced by hepatocytes (Parolini et al., 2010). AMSCs have been used in the treatment of acute chemical and thermal eye burns, pulmonary fibrosis, critical limb ischemia, inflammatory bowel disease, cardiac ischemia, and liver-based metabolic diseases, without any adverse side-effects (Parolini et al., 2010; Zhao et al., 2005; Meller et al., 2000). Another property of AMSCs that make them an attractive option for potential stem cell-based therapies is their low antigenicity. AMSCs express low levels of the highly polymorphic MHC class I antigens (HLA-A, HLA-B and HLA-C) but almost no MHC class II antigens (HLA-DP, HLA-DQ and HLA-DR) on their surfaces. This property is different from bone marrow-derived MSCs, that always display significant levels of MHC II antigens (Portmann-Lanz et al., 2006). Besides, MSCs are known to have the ability to modulate the function of several major immune cell types involved in alloantigen recognition and elimination, including T cells, B cells, natural killer cells, and antigen presenting cells (Shi et al., 2012).

Microglia are the major immunocompetent cells of the central nervous system (CNS), and strongly implicated in both neuroprotection as well as neurodegeneration. Under normal conditions, microglia act analogous to peripheral macrophages, as they secrete trophic factors and cytokines and remove debris and toxins from the extracellular space (Ni and Aschner, 2010). But during traumatic brain injury (TBI), or neurodegenerative diseases like Parkinson's disease and Alzheimer's disease, microglia are activated. These activated microglia respond to focal cerebral ischemic insults by migrating rapidly to the lesion sites, and releasing pro-inflammatory factors such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β, which are neurotoxic (Burguillos et al., 2011; Lambertsen et al., 2005). Activated microglia are also involved in facilitating other diseases of the CNS, including ischemia, infectious diseases, inflammatory demyelinating diseases, and neoplastic diseases (Tambuyzer et al., 2009). It has been demonstrated that blockade of microglial activation using anti-inflammatory drugs such as minocycline, can attenuate the pathology of Parkinson's disease (Wu et al., 2002).

In this study, we have analyzed the interactions of human AMSCs with human microglia and BV2 cells (an immortalized mouse microglial cell line infected with a v-raf/v-myc oncogene carrying retrovirus J2), to determine whether the former can influence the viability and migratory properties of microglia. Additionally, we have also explored possible mechanisms underlying the immunosuppressive effect of AMSCs on microglia.

2. Results

2.1. *Characterization of AMSC-morphology and cell surface markers*

We observed the morphology of P3 AMSCs under the microscope. At 24 h after seeding, AMSCs formed new colonies and exhibited a typical spindle-shaped cell body (Fig. 1A). Using flow cytometry, we analyzed the expression of different cell surface molecules, CD29, CD45, CD90 and CD11b, and found that while more than 95% of P3 AMSCs express AMSC-specific markers CD29 (99.38%) and CD90 (99.85%), they lacked the expression of hematopoietic surface markers CD45 (0.73%) and CD11b (2.19%) (Fig. 1B).

2.2. *AMSCs decrease the viability of human microglia and BV2 cells*

Previous studies have shown that MSCs can reduce the viability of a variety of immune cells. Based on these reports, we first tested the ability of AMSCs to influence the survival of the major immune cells of the CNS – the microglia. We used conditioned medium (CM) derived from AMSCs to culture human microglia and BV2 cells, and found that the growth of both these cell types decreased progressively in a time-dependent manner. Specifically, the viability of these cells decreased by 60.64±10.74% (human microglia) and 58.43±9.58% (BV2 cells) after 48 h in AMSCs conditioned medium (AMSC-CM) (Fig. 2A and B). These findings suggest that the immunosuppressive function of AMSCs might be mediated via its effect on the viability of immune cells such as the microglia, most likely by secreting certain extracellular soluble factors.

2.3. *AMSCs significantly decrease migration of human microglia and BV2 cells to wound site*

Microglia are highly migratory cells, and are known to migrate to lesions and injury sites in vivo. Hence, we next investigated whether AMSCs can affect this important property of microglia using the wound healing assay. As shown in Fig. 3A and C, control human microglia and BV2 cells spontaneously migrated and filled 62.37±10.94% and 72.78±12.64% of the wounded