Cytotherapy, 2016; 18: 630–641

International Society for Cellular Therapy
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UMBILICAL CORD CELLS

Toll-like receptor 3 pre-conditioning increases the therapeutic efficacy of umbilical cord mesenchymal stromal cells in a dextran sulfate sodium–induced colitis model

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Abstract

Background aims. Immunomodulatory properties of human umbilical cord–derived mesenchymal stromal cells (UCMSCs) can be differentially modulated by toll-like receptors (TLR) agonists. Here, the therapeutic efficacy of short TLR3 and TLR4 pre-conditioning of UCMSCs was evaluated in a dextran sulfate sodium (DSS)-induced colitis in mice. The novelty of this study is that although modulation of human MSCs activity by TLRs is not a new concept, this is the first time that short TLR pre-conditioning has been carried out in a murine inflammatory model of acute colitis. *Methods.* C57BL/6 mice were exposed to 2.5% dextran sulfate sodium (DSS) in drinking water *ad libitum* for 7 days. At days 1 and 3, mice were injected intraperitoneally with 1×10^6 UCMSCs untreated or TLR3 and TLR4 pre-conditioned UCMSCs. UCMSCs were pre-conditioned with poly(I:C) for TLR3 and LPS for TLR4 for 1 h at 37°C and 5% CO_2. We evaluated clinical signs of disease and body weights daily. At the end of the experiment, colon length and histological changes were assessed. *Results.* poly(I:C) pre-conditioned UCMSCs significantly ameliorated the clinical and histopathological severity of DSS-induced colitis compared with UCMSCs or LPS pre-conditioned UCMSCs. In contrast, infusion of LPS pre-conditioned UCMSCs significantly increased clinical signs of disease, colon shortening and histological disease index in DSS-induced colitis. *Conclusions.* These results show that short *in vitro* TLR3 pre-conditioning with poly(I:C) enhances the therapeutic efficacy of UCMSCs, which is a major breakthrough for developing improved treatments to patients with inflammatory bowel disease.

Key Words: *cell therapy, dextran sulfate sodium–induced colitis, immunosuppression, inflammatory bowel disease, toll-like receptors, umbilical cord–derived mesenchymal stromal cells*

Introduction

Mesenchymal stromal cells (MSCs) are plastic adherent, fibroblast-like multipotent, non-hematopoietic progenitor cells isolated from a variety of adult tissues, including bone marrow and adipose tissue, as well as in fetal tissues [1,2]. *In vitro* expanded MSCs have the potential to differentiate into tissues of mesodermal origin, such as adipocytes, chondroblasts and osteoblast under standard *in vitro* conditions providing a potential role in tissue repair [1,3]. Recently, MSCs have attracted much attention because they appear to exert potent *in vitro* and *in vivo* immunomodulatory effects via the suppression of T, B, natural killer (NK) and antigen/presenting cells [4,5]. Also, there is an emerging amount of data that indicates that MSCs escape recognition of allo-reactive cells and remain hypo-immunogenic [6,7]. In addition, MSCs do not appear to express the costimulatory molecules CD40, CD40L, CD80 or CD86 required for effector T-cell

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(Received 28 October 2015; accepted 4 February 2016)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2016.02.002

activation [8,9]. These immunomodulatory properties of MSCs, along with their non-tumorigenic nature and the ability for isolation and expansion *in vitro* without losing their phenotype or multi-lineage potential, have generated great interest for their potential as a promising therapeutic modality for immune-mediated diseases and tissue repair [10,11]. Not surprisingly, several groups have reported therapeutic effects of MSCs in experimental animal models of autoimmune and pro-inflammatory diseases such as experimental autoimmune encephalomyelitis (EAE) [12–14], collagen-induced arthritis (CIA) [15,16] experimental colitis [17,18] and also in human graft-versus-host disease [19] and systemic lupus erythematosus [20].

Despite the *in vitro* and *in vivo* evidence for the therapeutic effect of MSCs, their precise mechanism of action or their adverse effects profile as immunomodulatory agents are poorly understood. Several reports have evidenced that the suppressive effects of human MSCs involve both cell contact and soluble factors such as indoleamine 2,3-dioxygenase (IDO), transforming growth factor beta, interleukin (IL)-10 and prostaglandins, among others [5,21–23]. The net effect seems to inhibit proinflammatory Th1 and NK cells, while promoting anti-inflammatory Th2 and/or suppressive T regulatory (Treg) cells [24,25]. Although these observations suggest that MSCs could induce a shift from a pro-inflammatory to an anti-inflammatory state, others reports show opposite results *in vivo*. For example, various authors have demonstrated that administration of MSCs, in advanced stages of disease, tends to worsen CIA [26–28].

In accordance with these findings, our group has observed that murine MSCs show opposing effects on T-helper subsets (Th1, Th17 and Treg cells), according to the state of CD4 + T-cell activation. MSCs exhibit their typical suppressive effect on Th1, Th17 and Treg cells when added early to naive CD4 +cell cultures in the presence of Th CD4 + polarizing stimuli. However, late addition of MSCs, once T-cell activation has occurred, brings forth an unexpected stimulating effect on pro-inflammatory Th17 cells, leaving Treg cells unchanged [29].

These data underline the complexity of the MSC-mediated immunosuppressive effects and suggest that MSC function is critically responsive to the specific milieu and combination of pro-inflammatory/anti-inflammatory factors present *in vitro* or *in vivo*. Recently, studies have shown that the short stimulation of specific toll-like receptor (TLR)3 or TLR4 affects the immunomodulatory properties of MSCs. In fact, it has been described that human bone marrow–derived MSCs (BM-MSCs) polarize into either a pro-inflammatory or an immunosuppressive phenotype, according to the activation of specific and functionally different TLR [30]. Although the stimulation of TLR3 with poly(I:C) triggers immunosuppression, stimulation of TLR4 with LPS induces MSC production of IL-6 and IL-8, reversing immunosuppression. These results suggest that MSCs could behave as a "bi-functional" pro-inflammatory or immunosuppressive cell according to the specific signalling mediated by TLR3 and TLR4.

We decided, therefore, to demonstrate that *in vitro* pre-conditioning with poly(I:C) and LPS can induce two distinct active phenotypes in umbilical cord–derived MSCs (UCMSCs) and that these polarized cells possess opposite immunological effects in a mouse model of dextran sulfate sodium (DSS)-induced colitis. To our knowledge, this is the first time that short TLR3 and TLR4 pre-conditioning of MSCs has been studied for DSS-induced colitis, an experimental model of intestinal inflammation. In this study, we demonstrate that short *in vitro* TLR3 pre-conditioning enhances the therapeutic efficacy of UCMSCs in DSS-induced colitis, which can be very useful for treatment or amelioration of inflammatory bowel diseases (IBD) or other pro-inflammatory diseases that affect the general population.

Methods

Mice

Eight- to 10-week-old female C57BL/6 mice were purchased from Central Animal Facility, Public Health Institute of Chile. Animals were housed under standard laboratory conditions and given food and water *ad libitum*. Mice were allowed to acclimate to these conditions for at least 21 days before inclusion in experiments. Experimental procedures and protocols were performed according to the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996) and approved by the Institutional Animal Care and Use Committee of the University of the Andes Faculty of Medicine and by the Chilean Fondecyt Bioethics Advisory Committee.

Isolation and culture of UCMSCs

Human MSCs from umbilical cords were kindly provided by Cells for Cells S.A. (Santiago, Chile). Briefly, MSCs were isolated from fresh umbilical cords obtained from healthy mothers after normal deliveries, with their informed consent. The umbilical cords were rinsed carefully to remove cord blood in complete cell culture medium consisting of Dulbecco's Modified Eagle's Medium (Gibco, Life Technologies) supplemented with 10% heat-inactivated MSC-qualified fetal bovine serum (FBS, Gibco), 2 mmol/L glutamine (Biological Industries), 100 U/mL penicillin and 100 µg/