EXPERIMENTAL CELL RESEARCH 324 (2014) 65–74

Available online at www.sciencedirect.com

ScienceDirect

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

Research Article

Immunomodulation of mesenchymal stromal cells on regulatory T cells and its possible mechanism

Zhidong Yan[a], *Yongxun Zhuansun*[a], *Rui Chen*[a], *Jianguo Li*[a,*], *Pixin Ran*[b]

[a]*Department of Respiratory Medicine, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou 510120, Guangdong Province, China*

[b]*The State Key Laboratory of Respiratory Disease, the First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, Guangdong Province, China*

ARTICLE INFORMATION

Article Chronology:
Received 13 November 2013
Received in revised form
13 March 2014
Accepted 17 March 2014
Available online 25 March 2014

Keywords:
Mesenchymal stromal cells
Regulatory T cells
Programmed cell death 1 receptor
Immunomodulation

ABSTRACT

Mesenchymal stromal cells (MSCs) and regulatory T cells (Tregs) have both garnered abundant interests from immunologists worldwide, as both MSCs and Tregs can be considered immunosuppressive in their own right. But a little attention has been paid to the impacts of MSCs on Tregs. To clarify the effects of MSCs on Tregs, we performed the coculture systems within MSCs and Tregs. We confirmed that MSC-exposed Tregs are capable of more immunosuppressive than Tregs without coculturing with MSCs. And this augmenting suppressive capacity was accompanied with an upregulation of programmed cell death 1 receptor (PD-1) on Tregs. Importantly, we found that cell viability of Tregs was excluded from the influences of MSCs. Finally, we showed that PD-1/B7-H1 interactions and IL-10 might be responsible for the enhanced suppressive capability of MSC-exposed Tregs. Further analysis revealed that PD-1/B7-H1 interactions were not responsible for the productions of IL-10 and TGF-β_1 in the MSC-Treg coculture systems; in contrast, IL-10 rather than TGF-β_1 played a role in the upregualtion of PD-1. Furthermore, this is the first explorative study to evaluate the immunomodulation of MSCs on the suppressive capacity of Tregs in MSC–Treg in vitro coculture setting.

Introduction

Few cell types have captivated so many biomedical researchers over the last 10 years as have mesenchymal stromal cells (MSCs). It is well known that MSCs exert an immune regulatory function and regulate immune responses through multiple redundant pathways [1]. Although a large amount of studies have documented the immunosuppressive activities of MSCs [2–7] and their potential therapeutic usage in animals and humans [8–11], the underlying mechanisms are only partially known. In a dose-dependent manner, contact-dependent mechanisms and soluble factors are thought to collaborate for the induction of MSC-mediated immunosuppression. Several soluble immunosuppressive factors have been reported to be involved in MSC-

Abbreviations: MSCs, mesenchymal stromal cells; Tregs, regulatory T cells; PD-1, programmed cell death 1 receptor; Tconv, conventional $CD4^+$ $CD25^-$ T cells

*Corresponding author. Fax: +86 2081332960.
E-mail address: jgli88@126.com (J. Li).

http://dx.doi.org/10.1016/j.yexcr.2014.03.013
0014-4827/

mediated immunoregulation, either produced constitutively by MSCs or released following cross-talk with target cells. Such as nitric oxide (NO), indoleamine 2,3-dioxygenase (IDO), transforming growth factor-β (TGF-β), hepatocyte growth factor (HGF), PGE_2, IL-10, IL-6, heme oxygenase-1 (HO-1) and soluble HLA-G5 [1].

It is also commonly accepted that immunosuppression can be accomplished by lymphocyte populations termed regulatory T cells (Tregs) [12–16]. The underlying mechanisms involving the direct interactions of a given regulatory subset and responder lymphocytes and/or the release, by the regulatory cells of immune regulating cytokines including TGF-β or IL-10 which inhibit lymphocyte response, have gained a consistent approval [13–16]. Despite their ability to suppress immune responses by soluble factors, it is well documented that cell–cell contact manner utilized by Tregs suppression including CTLA-4 and LAG-3 [17]. Another molecule that is proposed to be involved in the cell-contact dependent immunosuppression is the programmed death-1 receptor (PD-1). PD-1 is expressed on TCR ligation on activated Tregs, but also on conventional $CD4^+$ $CD25^-$ T cells (Tconv) though in lower amounts [18]. Humans and animals with a deficiency in PD-1 expression are susceptible to autoimmune diseases [19,20]. It is worth noting that, $PD\text{-}1^{-/-}$ T cells have been found to be incapable to control autoimmunity. Moreover, blocking of PD-1/B7-H1 interplays has been shown to accelerate the outcome of several autoimmune diseases, such as diabetes, EAE, and colitis [21,22]. Similarly, PD-1/B7-H1 interactions are shown closely related to the suppression of antiviral T cell activity [23]. In general, PD-1/B7-H1 pathway is expected to be another regulatory mechanism related to immunosuppressive activities in the periphery.

In recent years, MSCs and Tregs have both garnered abundant interests from immunologists worldwide, particularly because of the potential application of both cell types in the treatment of many chronic inflammatory and autoimmune diseases. Although both MSCs and Tregs can be considered immunosuppressive in their own right, lots of previous studies have emphasized the relationship between MSCs and Tconv, the correlation between MSCs and Tregs is now obtained relatively through small number of investigation. Indeed, it is becoming increasingly apparent that there exist complex interactions between MSCs and Tregs [24].

In our current report, we investigated the immunomodulatory roles of MSCs on the regulation of Tregs phenotype and suppressive capability. Our results showed that significant PD-1 upregulation and elevated productions of IL-10 and TGF-β_1 were observed after coculturing MSCs with Tregs. These changes were combined with a significantly enhanced suppressive capacity of Tregs which might be partially mediated by the PD-1/B7-H1 interactions and IL-10. Thus, the capability of MSCs to enhance Tregs function may be an important mechanism to augment the immunosuppressive activities of Tregs in the potential cell therapy.

Materials and methods

Cells and cell culture

MSCs from human bone marrow (passage 2, accompanied with authentication reports) were obtained from Cyagen Biosciences Inc. (Guangzhou, China) and derived originally from 3 healthy adults from 18 to 45 years old. The cells were cultured in human mesenchymal stem cell growth medium with serum-free (Biowit Technologies, Shenzhen, China). Medium was changed every 2–3 days. After confluence (80–90%) was attained, the cells were detached by mild treatment with trypsin (0.25% [w/v], 1–2 min, 37 °C) and replated at one-third of the confluent density for continued passage. MSCs before the eighth subculture were used in the experiments. Human peripheral blood mononuclear cells (PBMCs) were isolated with Histopaque-1077 (Sigma-Aldrich, St. Louis, MO, USA) density centrifugation from leukapheresis products of more than 3 healthy donors (obtained from the Guangzhou Blood Center, China, after official authorization was given). All experiments included MSCs from 3 donors and Tregs from another 3 donors at least, and each independent experiment was analyzed in triplicates.

Purification of $CD4^+CD25^-$ Tconv and $CD4^+CD25^+$ Tregs

Separation of Tregs ($CD4^+CD25^+$ T cells) and Tconv ($CD4^+CD25^-$T cells) were performed using the human $CD4^+CD25^+$ regulatory T cell isolation kit and the AutoMACS separating system (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. The cells were labeled with anti-CD4-FITC, anti-CD25-APC and routinely analyzed by flow cytometry, the purity of Tregs and Tconv was >96%. Tregs and Tconv were cultured in RPMI 1640 (Gibco, Invitrogen Corporation. CA, USA) supplemented with 10% (v/v) FCS (Hyclone, Logan, UT, USA), 1% penicillin/streptomycin (v/v) (Hyclone), 1% HEPES (v/v) (Hyclone), and 5 nM β-mercaptoethanol (v/v) (Sigma-Aldrich, St. Louis, MO, USA). For all experiments, the regulatory function of Tregs was analyzed by their ability to suppress proliferation of Tconv in the presence of 0.5 μg/ml soluble anti-human CD3 and CD28 mAbs (BD Bioscience, Heidelberg, Germany).

MSC/Treg-cell cocultures

Allogeneic MSCs (1×10^5) were seeded on a 24-well culture plate (Corning, Amsterdam, The Netherlands) and incubated for 72 h. The prestimulated Tregs were directly or indirectly loaded by using transwell systems (Corning) onto MSCs and cocultured for 24 h. Tregs were isolated as described previously. Immediately after isolation, 1×10^5/ml Tregs were activated with 10 μg/ml plate-bound anti-human CD3 mAb and 0.5 μg/ml soluble anti-human CD28mAb (R&D Systems, Wiesbaden, Germany). After 24 h, the cells were harvested and washed thoroughly. The 1×10^5 activated Tregs were cocultured with 1×10^5MSCs in RPMI 1640-based complete medium. After 24 h cocultures, Tregs were harvested and culture supernatants were collected for further analysis. For contact-independent cocultures, 1×10^5 Tregs were cultured in 0.4 μm transwell inserts and 1×10^5MSCs were cultured in the lower chambers. For neutralization experiments, cocultures were performed in the presence of 10 μg/ml anti-human TGF-β_1 mAb (R&D Systems) or 10 μg/ml anti-human IL-10 mAb (R&D Systems). In parallel, isotype-matched control mAbs were used to assess specificity of the neutralizing mAb. Complete neutralization of cytokines was assessed by ELISA of culture supernatants. For blocking of PD-1/B7-H1 interactions during cocultures, MSCs were incubated with 10 μg/ml anti-B7-H1 (Miltenyi Biotec, Bergisch Gladbach, Germany) or isotype control mAb prior coculture with 1×10^5Tregs.