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Mesenchymal stromal cell exosome–enhanced regulatory T-cell production through an antigen-presenting cell–mediated pathway

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Abstract

Background aims. The immunomodulatory property of mesenchymal stromal cell (MSC) exosomes is well documented. On the basis of our previous report that MSC exosomes increased regulatory T-cell (Treg) production in mice with allogenic skin graft but not in ungrafted mice, we hypothesize that an activated immune system is key to exosome-mediated Treg production. *Methods.* To test our hypothesis, MSC exosomes were incubated with mouse spleen $CD4^+$ T cells that were activated with either anti-CD3/CD28 mAbs or allogenic antigen-presenting cell (APC)-enriched spleen $CD11c^+$ cells to determine whether production of mouse $CD4^+CD25^+$ T cells or $CD4^+CD25^+Foxp3^+$ Tregs could be induced. MSC exosomes were also administered to the lethal chimeric human-SCID mouse model of graft-versus-host disease (GVHD) in which human peripheral blood mononuclear cells were infused into irradiated NSG mice to induce GVHD. *Results.* We report here that MSC exosome–induced production of $CD4^+CD25^+$ T cells or $CD4^+CD25^+Foxp3^+$ Tregs from $CD4^+$ T cells activated by allogeneic APC-enriched $CD11C^+$ cells but not those activated by anti-CD3/CD28 mAbs. This induction was exosome- and APC dose–dependent. In the mouse GVHD model in which GVHD was induced by transplanted human APC-stimulated human anti-mouse $CD4^+$ T cell effectors, MSC exosome alleviated GVHD symptoms and increased survival. Surviving exosome-treated mice had a significantly higher level of human $CD4^+CD25^+CD127^{low/-}$ Tregs than surviving mice treated with Etanercept, a tumor necrosis factor inhibitor. *Conclusions.* MSC exosome enhanced Treg production *in vitro* and *in vivo* through an APC-mediated pathway.

Key Words: *antigen-presenting cell, exosome, mesenchymal stromal cell, graft-versus-host disease, regulatory T cells*

Introduction

Mesenchymal stromal cells (MSCs) are multipotent fibroblast-like cells that could be readily isolated from a wide variety of tissues. They also have a large capacity for *ex vivo* expansion and are reported to have the potential to differentiate into all the three lineages: ectoderm, mesoderm and endoderm (reviewed in Ullah *et al.*) [1]. MSCs are also well known for their immunomodulatory potency [2]. MSCs are currently in clinical testing for many immune diseases [3], such as steroid-resistant graft-versus-host disease (GVHD) [4,5], Crohn disease [6,7] and multiple sclerosis [8]. In a landmark multicenter nonrandomized trial led by Katarina Le Blanc and colleagues from the European Group for Blood and Marrow Transplantation Mesenchymal Stem Cell Expansion Consortium, MSC transplantation induced complete responses in 55% of 55 patients with acute GVHD grade 2–4, and this response was independent of whether the MSCs were from third-party mismatched donors, human leukocyte antigen–identical siblings or haplo-identical family members [9].

Although MSC transplantation is effective in ameliorating GVHD, it does not impair graft-versus-leukemia (GVL) reactions [10,11], and this phenomenon has been attributed in part to MSC-mediated induction of regulatory T cells (Tregs) [12]. Tregs are a subpopulation of T cells known to attenuate immune activity and abrogate autoimmune diseases and as such have been implicated as a key immune cell type in the modulation of GVHD [13]. Tregs are progressively lost during GVHD in experimental murine models and patients [14–17]. *Ex vivo* infusion of Tregs suppressed GVHD but not GVL responses [18], whereas depletion of Tregs increased GVHD [19,20]. The hypothesis that MSCs' efficacy against GVHD is due to MSC-mediated induction of

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Tregs is supported by numerous lines of evidence. MSC infusion in mice after heart, islet or kidney transplant was reported to increase Tregs and increase survival of the transplanted tissues [21–23]. MSCs also induce Tregs in mouse models of experimental colitis, allergen-driven airway inflammation and autoimmune uveitis [24–26]. In a mouse model of collagen-induced arthritis, allogenic MSCs induce antigen-specific Tregs and prevent tissue damage [27]. Significantly, MSC infusion was found to be safe and enhance Treg level in a clinical study of two kidney transplant patients [28]. More recently, a phase II multicenter, randomized, double-blind study has reported that MSC infusions in chronic GVHD (cGVHD) patients increase memory B lymphocytes and Tregs and Th1:Th2 ratio [29].

The induction of Tregs by MSCs has been proposed to be mediated by MSC secretion as co-incubation of MSCs with $CD4^+$ T lymphocytes in a transwell system was sufficient in inducing generation of Tregs [24,30]. Consistent with this, MSCs have been shown to secrete many factors known to be important in the induction of Tregs, such as interleukin (IL)-10, transforming growth factor (TGF)-β and prostaglandin $(PGE)_2$. However, as previously discussed in our review, we have rationalized that in these factors secreted by MSC, exosomes represent the most plausible secreted candidate in mediating the induction of Tregs [31].

MSC exosomes are 100- to 200-ηm endosome-derived vesicles [32] with a protein- [33] and RNA-rich [34] cargo and were first reported when they were shown to reduce reperfusion injury in a mouse of acute myocardial ischemia [35–37]. Our group has demonstrated through pulse chase studies that our $CD9^+$, $CD81^+$ extracellular vesicles are endosome-derived [32] and are therefore bona fide exosomes. Since then, we have reported that MSC exosomes are also immunologically active [38] and could induce Tregs *in vitro* and *in vivo* [38]. They do not express MHC class I or II or co-stimulatory molecules such as CD40, CD80 and CD86 and can polarize THP-1 cells and primary mouse or human monocytes toward an M2 macrophage-like phenotype with elevated expression of anti-inflammatory IL-10 and an attenuated expression of pro-inflammatory (e.g., IL-1β, IL-6, tumor necrosis factor [TNF]-α, IL-12p40) genes. When pretreated with exosomes, THP-1 cells can induce Treg differentiation. In mice with allogeneic skin grafts, infusion of MSC exosomes increased Tregs and enhanced survival of allogenic skin graft. However, in nongrafted animals, infusion of MSC exosomes have no effect on Treg production. We hypothesize that MSC exosomes are immunologically modulatory only in the context of an activated immune response and not in a homeostatic immune system.

In this report, we tested this hypothesis by evaluating the capacity of MSC exosomes to induce Tregs *in vitro* using naive $CD4^+$ T cells that had been activated by anti-CD3 and CD28 antibody. MSC exosomes had minimal effects on the polarization of the activated $CD4^+$ T cells into $CD4^+CD25^+$ $Foxp3^-$ T cells. However, in the presence of allogenic $CD11C^+$ APCs, MSC exosomes induced significant polarization of naïve $CD4^+$ T cells to $CD4^+CD25^+Foxp3^+$ Treg in a dose-dependent manner. Infusion of MSC exosomes led to a significant improvement in mouse model of xenogeneic GVHD symptoms and reduction in mortality with an increase of human $CD4^+CD25^+CD127^{low/-}$ Tregs in a mouse model of GVHD induced by human peripheral blood mononuclear cells (PBMCs).

Methods

Mice

Six- to 8-week-old female BALB/c ($H\text{-}2^d$) and C57BL/6 ($H\text{-}2^b$) mice were purchased from InVivos Pte Ltd (Singapore) and housed in a specific pathogen-free facility. The 6- to 8-week-old female NSG mice were provided from Jackson Laboratory, as an outsourced service of an *in vivo* experiment to assess the effect of a novel therapeutic of MSC exosomes on survival in a model of human PBMC-induced GVHD (with irradiation). All manipulations were performed according to the protocols approved by the Institutional Animal Care and Use Committee.

Preparation of MSC exosomes

MSC exosome was prepared as previously described [35,36,39]. Briefly, immortalized human embryonic stem cell–derived MSCs were grown in a chemically defined medium for 3 days and the conditioned medium was harvested and 0.22-mm filtered. The conditioned medium was concentrated 100 × for exosomes by tangential flow filtration (Sartorius; MWCO 100 kDa) and stored in −20°C freezer until use. The exosomes were assayed for protein concentration using Coomassie Plus (Bradford) Assay (Thermo Fisher Scientific), as per manufacturer's instruction, and characterized for particle size distribution and concentration by Zetaview (Particle Metrix) according to the manufacturer's protocol. The mean protein and particle contents of the exosome preparations were 1.9 mg/mL and 1.9×10^{11} particles/mL.

Incubation of MSC exosomes with $CD4^+$ T cells activated by anti-cd3cd28 mAb

$CD4^+$ T cells were isolated from C57BL/6 mouse spleens as previously described [38]. Briefly, the spleens