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Transplantation of skin mesenchymal stem cells attenuated AngII-induced hypertension and vascular injury

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ABSTRACT

Skin mesenchymal stem cells (S-MSCs) revealed an important immunomodulatory activity to markedly suppress the formation of the atherosclerosis (AS) plaque by modulating macrophages, and also inhibit the development of experimental autoimmune encephalomyelitis (EAE) by regulating T helper 17 (Th17) cell differentiation. Macrophages and Th17 cells play important roles in hypertension. However, it remains unclear whether S-MSCs are capable of improving angiotensin (AngII)-induced hypertension by acting on inflammatory cells. Therefore, we studied a direct effect of S-MSC treatment on an AngII-induced hypertensive mouse model. Twenty-seven C57BL/6 (WT) mice were divided into three groups: Control group (WT-NC), AngII-infused group (WT-AngII), and S-MSC treatment group (WT-AngII + S-MSCs). In contrast to WT-AngII group, systolic blood pressure (SBP) and vascular damage were strikingly attenuated after tail-vein injection of S-MSCs. Numbers of Th17 cells in mouse peripheral blood of S-MSC treated group were significantly decreased, and IL-17 mRNA and protein levels were also reduced in the aorta and serum compared with WT-AngII group. Furthermore, macrophages in S-MSC treated group were switched to a regulatory profile characterized by a low ability to produce pro-inflammatory cytokine TNF-α and a high ability to produce anti-inflammatory cytokines Arg1 and IL-10. Mechanistically, we found that S-MSCs inhibited Th17 cell differentiation and induced M2 polarization. Moreover, we found proliferation and migration of S-MSCs were elevated, and expression of CXCR4, the receptor for Stromal derivated factor −1(SDF-1), was markedly increased in lipopolysaccharide (LPS)- stimulated S-MSCs. Given that SDF-1 expression was increased in the serum and aorta in AngII- induced hypertensive mice, the immunomodulatory effects exerted by S-MSCs involved the CXCR4/SDF-1 signaling. Collectively, our data demonstrated that S-MSCs attenuated AngII-induced hypertension by inhibiting Th17 cell differentiation and by modulating macrophage M2 polarization, suggesting that S-MSCs potentially have a role in stem cell based therapy for hypertension.

1. Introduction

Skin mesenchymal stem cells (S-MSCs), a novel population of MSCs, with functional similarities to bone marrow-derived MSCs (BM-MSCs), are a promising cell source for stem cell-based therapies of chronic inflammatory diseases and possibly transplantation [1]. In recent studies, S-MSCs displayed a remarkable immunosuppressive activity to suppress the development of atherosclerosis [2] and EAE [3] in mouse models.

Emerging evidence showed that both innate and adaptive immune responses play an important role in the pathogenesis of hypertension and vascular damage [4,5]. Studies have confirmed that the increased numbers of T cells infiltration and augmented T-cell–derived cytokines such as interleukin 17A (IL-17A), interferon-gamma (IFN-γ), and tumor necrosis factor-α (TNF-α) in the adventitia and Perivascular Adipose Tissue (PVAT) contribute to AngII-induced organ injury and hypertension [6,7]. Recent evidence supports that specific subsets of T cells (Th17, Th1, and Treg) are important in hypertension [8–10]. Modulation of T cell

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differentiation and the secreted cytokines have improved hypertension [10]. Th17 cell and IL-17 played a critical pathophysiological role and served as therapeutic targets for the treatment of hypertension and vascular damage [9]. Recurrent data have demonstrated that the administration of S-MSCs was able to inhibit Th17 cell differentiation and attenuate the development of EAE [3]. Therefore, inhibition of Th17 cell differentiation by S-MSCs may be a feasible strategy for the intervention of hypertension and vascular damage.

Macrophage infiltration and the production of the majority of inflammatory cytokines in the damaged organ played a critical role in the pathophysiology of hypertension [11]. Reduction of macrophage infiltration [12,13] and induction of macrophage M2 polarization [14] played a central role in the pathogenesis of hypertension. Our study has displayed that S-MSCs could release COX-2- dependent PGE2 (increased production in vascular tissue of hypertension mice) [15] acting on macrophage receptors EP2 and EP4, stimulating macrophage M2 polarization to inhibit the formation of the AS plaque [2]. However, there is no evidence implicating the effect of S-MSC-induced macrophage M2 polarization in AngII- induced hypertension.

S-MSCs have the ability to migrate to the inflamed tissues in AS mice [2]. SDF-1, termed CXCL12, is not only involved in the immune system but also in migration of cancer cells, and extracellular matrix adhesion of haematopoietic cells in the bone marrow or damaged tissues [16,17]. The vitro results suggested that the SDF-1/CXCR4 axis could induce recruitment of expanded MSCs to damaged tissues [18]. Ocular administration of SDF-1 in the rat increases intraocular pressure [17]. Levels of SDF-1 in plasma were increased comparing patients with essential hypertension to healthy people. We found that activated S-MSCs produced high amount of CXCR4. Thus, S-MSCs injected to the hypertensive mice may directly migrate to the damaged organs and be activated to perform their suppressive function.

Here, we demonstrated that administration of S-MSCs was able to attenuate AngII- induced hypertension by inhibiting Th17 cell differentiation and modulating macrophage M2 polarization. We further found that inflammation elevated the proliferation and migration of S-MSCs and activated S-MSCs to produce high amount of CXCR4, which is the receptor for SDF-1 increased in the serum and aorta of AngII- induced hypertension mice, suggesting that S-MSCs may have therapeutic potential for the treatment of hypertension.

2. Materials and methods

2.1. Mice

C57BL/6 mice were purchased from the Animal Center of Peking University, Beijing, China (approval number: SCXK [Jing] 2006-0008). All mice were kept under specific pathogen-free conditions in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals with the approval (SYXK-2003-0026) of Scientific Investigation Board of Shanghai Jiao Tong University School of Medicine, Shanghai, China. Starting from 8 to 10 weeks of age, 27 male C57BL/6 mice were randomly divided into 3 groups: Control group (WT-NC, infused with 0.9% NaCl), AngII-infused group (WT-AngII, infused with AngII for 28 days- 750 ng/kg per minute), S-MSC treatment group (WT-AngII + S-MSC, infused with AngII and received 0.5 million S-MSCs weekly tail-vein injection at day 6 AngII infused for the final 21 days). Minipumps (1004, Alzet, DURECT Corporation) were implanted subcutaneously in mice to deliver AngII (Sigma) or 0.9% NaCl. Systolic blood pressure (SBP) was measured by tail-cuff by using BP-2000 Blood Pressure Analysis System (Visitech Systems, Napa Place Apex, NC).

2.2. Cells isolation, culture, and differentiation

Preparation and culture of S-MSCs were performed as previously [2].

Peripheral blood T cells were isolated using a mouse Pan T Cell Isolation Kit II (negative selection, Miltenyi Biotech, each sample was from two mice Peripheral blood), which were stimulated by phorbolmyristate acetate (PMA, 50 ng/ml, sigma)/ionomycin (1000 ng/ml, Sigma) in the presence of the GolgiPlug (1000×, BD) for 5 h. Naive $CD4^+$ T cells isolated from mouse spleen by MagniSort® mouse CD4 Naive T cells Enrichment kit (Invitrogen) and Th17 cells differentiation conditions were described as before [19].

2.3. Histology and immunofluorescence

Aorta tissue fixed by Paraformaldehyde was embedded in paraffin and 6-μm sections were stained with HE or Masson Trichrome staining [2]. For immunofluorescence staining, the cultured S-MSCs were stained with anti-mouse CXCR4 (UMB2-ab124824, Abcam), and Alexa555-conjugated goat anti-Rabbit IgG1 (Invitrogen) was as secondary antibody, 4′,6-diamidino-2-phenylindole (DAPI) (Sigma) was used for nuclei staining.

2.4. Flow cytometry

Anti-Mouse $CD4^-$ APC and IL-17A-PE antibodies were used and from eBioscience. Intracellular staining of IL-17A: Peripheral blood T cells stimulated with PMA and ionomycin for 5 h in the presence of the GolgiPlug, or vitro differentiated Th17 cells were incubated with APC-conjugated anti-CD4 antibody. After fixation and permeabilization, cells were stained with anti-IL-17A-PE.

2.5. ELISA

Quantitative analysis of IL-17 and SDF-1 in serum of mouse was performed by ELISA using commercially available kits (Mouse IL-17 (IL-17A) ELISA Kit, Sigma; SDF-1 alpha Mouse ELISA kit, Abcam).

2.6. Quantitative real-time-polymerase chain reaction (RT-PCR)

Total RNA was extracted from mouse tissue or cultured cells with TRIzol Reagent (Invitrogen). Reverse transcription was performed as previously [20]. Quantitative PCR was carried out using the SYBR Green PCR Master Mix (TaKaRa) in the ABI Prism 7900 (Applied Biosystems). All gene expression results were normalized to the expression of the house keeping gene GAPDH. Primer sequences were as follows: IL-17A (forward:5′-GCTCCAGAAGGCCCTCAGA-3’; reverse:5′-CTTTCCCTCCGCATTGACA-3′); TNF-a (forward:5′-GAACTGGCAGAAGAGGCACT-3’; reverse: 5′-AGGGTCTGGGCCATAGAACT-3′); IL-10 (forward:5′- CAGCCGGGAAGACAATAACT-3’; reverse: 5′-GCATTAAGGAGTCGGTTAGCA-3′); SDF-1(forward:5′- TTGTCTCAACCCTGAAGCCC-3’; reverse:5′-TGCCCGTTGAGGTACAGGAG-3′); F4/80 (forward: 5′-AGGACTGGAAGCCCATAGCCAA-3’; reverse:5′-GCATCTAGCAATGGACAGCTG-3′); Arg1(forward: 5′-GGAGAGCCTTCCTGCACTTT-3’; reverse: 5′-GTGCCTTGGTCTACATTGAACATAC-3’); GAPDH (forward:5′-TGTGTCCGTCGTGGATCTGA-3’; reverse:5′-CCTGCTTCACCACCTTCTTGA-3′).

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