



11-2011

## Human Gingiva-Derived Mesenchymal Stromal Cells Attenuate Contact Hypersensitivity via Prostaglandin E2- Dependent Mechanisms

Wen-Ru Su


Qun-Zhou Zhang  
*University of Pennsylvania*

Shi-Hong Shi  
*University of Pennsylvania*

Andrew L. Nguyen

Anh D. Le  
*University of Pennsylvania*

Follow this and additional works at: [https://repository.upenn.edu/dental\\_papers](https://repository.upenn.edu/dental_papers)

 Part of the [Endodontics and Endodontology Commons](#), [Oral and Maxillofacial Surgery Commons](#), and the [Oral Biology and Oral Pathology Commons](#)

---

### Recommended Citation

Su, W., Zhang, Q., Shi, S., Nguyen, A. L., & Le, A. D. (2011). Human Gingiva-Derived Mesenchymal Stromal Cells Attenuate Contact Hypersensitivity via Prostaglandin E2- Dependent Mechanisms. *Stem Cells*, 29 (11), 1849-1860. <http://dx.doi.org/10.1002/stem.738>

At the time of publication, author Qun-Zhou Zhang was affiliated with the University of Southern California.

Currently, (s)he is a faculty member at the School of Dental Medicine at the University of Pennsylvania.

At the time of publication, author Shihong Shi was affiliated with the University of Southern California. Currently, (s)he is a faculty member at the School of Dental Medicine at the University of Pennsylvania.

At the time of publication, author Anh D. Le was affiliated with the University of Southern California. Currently, (s)he is a faculty member at the School of Dental Medicine at the University of Pennsylvania.

This paper is posted at ScholarlyCommons. [https://repository.upenn.edu/dental\\_papers/539](https://repository.upenn.edu/dental_papers/539)

For more information, please contact [repository@pobox.upenn.edu](mailto:repository@pobox.upenn.edu).

---

# Human Gingiva-Derived Mesenchymal Stromal Cells Attenuate Contact Hypersensitivity via Prostaglandin E2- Dependent Mechanisms

## Abstract

The immunomodulatory and anti-inflammatory functions of mesenchymal stromal cells (MSCs) have been demonstrated in several autoimmune/inflammatory disease models, but their contribution to the mitigation of contact hypersensitivity (CHS) remains unclear. Here, we report a new immunological approach using human gingiva-derived MSCs (GMSCs) to desensitize and suppress CHS and the underlying mechanisms. Our results showed that systemic infusion of GMSCs before the sensitization and challenge phase dramatically suppress CHS, manifested as a decreased infiltration of dendritic cells (DCs), CD8 + T cells, T H-17 and mast cells (MCs), a suppression of a variety of inflammatory cytokines, and a reciprocal increased infiltration of regulatory T cells and expression of IL-10 at the regional lymph nodes and the allergic contact areas. The GMSC-mediated immunosuppressive effects and mitigation of CHS were significantly abrogated on pretreatment with indomethacin, an inhibitor of cyclooxygenases. Under coculture condition of direct cell-cell contact or via transwell system, GMSCs were capable of direct suppression of differentiation of DCs and phorbol 12-myristate 13-acetate-stimulated activation of MCs, whereas the inhibitory effects were attenuated by indomethacin. Mechanistically, GMSC-induced blockage of de novo synthesis of proinflammatory cytokines by MCs is mediated partly by the tumor necrosis factor-alpha/prostaglandin E 2 (PGE 2) feedback axis. These results demonstrate that GMSCs are capable of desensitizing allergic contact dermatitis via PGE 2-dependent mechanisms. © AlphaMed Press.

## Keywords

Author keywords: Contact hypersensitivity, Human gingival, Immunomodulation, Mast cells, Stromal cells  
MeSH: Animals, Cells, Cultured, Dermatitis, Contact, Dinoprostone, Flow Cytometry, Gingiva, Humans, Mesenchymal Stromal Cells, Mice  
EMTREE drug terms: interleukin 10, prostaglandin E2, tumor necrosis factor alpha  
EMTREE medical terms: animal experiment, animal model, animal tissue, article, CD8+ T lymphocyte, cell differentiation, cell interaction, contact allergy, controlled study, dendritic cell, desensitization, gingiva derived mesenchymal stromal cell, human, human cell, human tissue, immunomodulation, intracellular signaling, lymph node, lymphocytic infiltration, male, mast cell, mesenchymal stem cell, mouse, nonhuman, protein expression, regulatory T lymphocyte, stroma cell, Th17 cell

## Disciplines

Dentistry | Endodontics and Endodontology | Oral and Maxillofacial Surgery | Oral Biology and Oral Pathology

## Comments

At the time of publication, author Qun-Zhou Zhang was affiliated with the University of Southern California. Currently, (s)he is a faculty member at the School of Dental Medicine at the University of Pennsylvania.

At the time of publication, author Shihong Shi was affiliated with the University of Southern California. Currently, (s)he is a faculty member at the School of Dental Medicine at the University of Pennsylvania.

At the time of publication, author Anh D. Le was affiliated with the University of Southern California. Currently, (s)he is a faculty member at the School of Dental Medicine at the University of Pennsylvania.

# Human Gingiva-Derived Mesenchymal Stromal Cells Attenuate Contact Hypersensitivity via Prostaglandin E2-Dependent Mechanisms

Wen-Ru Su, Qun-Zhou Zhang, Shi-Hong Shi, Andrew L. Nguyen, Anh D. Le



The advertisement banner features a dark blue background on the left with a white control panel on a piece of equipment. The text is arranged as follows: "You Don't Need Reproducible Research" in green, "UNTIL YOU DO." in white, "Minimize uncertainty with PHCbi brand products" in white on a green bar, and the "phcbi" logo in blue on the right.

You Don't Need Reproducible Research  
**UNTIL YOU DO.**  
Minimize uncertainty with PHCbi brand products  
**phcbi**

## Human Gingiva-Derived Mesenchymal Stromal Cells Attenuate Contact Hypersensitivity via Prostaglandin E<sub>2</sub>-Dependent Mechanisms

WEN-RU SU, QUN-ZHOU ZHANG, SHI-HONG SHI, ANDREW L. NGUYEN, ANH D. LE

Center for Craniofacial Molecular Biology, The Herman Ostrow School of Dentistry of University of Southern California, Los Angeles, California, USA

**Key Words.** Human gingival • Stromal cells • Immunomodulation • Contact hypersensitivity • Mast cells

### ABSTRACT

The immunomodulatory and anti-inflammatory functions of mesenchymal stromal cells (MSCs) have been demonstrated in several autoimmune/inflammatory disease models, but their contribution to the mitigation of contact hypersensitivity (CHS) remains unclear. Here, we report a new immunological approach using human gingiva-derived MSCs (GMSCs) to desensitize and suppress CHS and the underlying mechanisms. Our results showed that systemic infusion of GMSCs before the sensitization and challenge phase dramatically suppress CHS, manifested as a decreased infiltration of dendritic cells (DCs), CD8<sup>+</sup> T cells, T<sub>H</sub>-17 and mast cells (MCs), a suppression of a variety of inflammatory cytokines, and a reciprocal increased infiltration of regulatory T cells and expression of IL-10 at the regional lymph nodes and the allergic contact areas. The GMSC-mediated immu-

nosuppressive effects and mitigation of CHS were significantly abrogated on pretreatment with indomethacin, an inhibitor of cyclooxygenases. Under coculture condition of direct cell-cell contact or via transwell system, GMSCs were capable of direct suppression of differentiation of DCs and phorbol 12-myristate 13-acetate-stimulated activation of MCs, whereas the inhibitory effects were attenuated by indomethacin. Mechanistically, GMSC-induced blockage of de novo synthesis of proinflammatory cytokines by MCs is mediated partly by the tumor necrosis factor-alpha/prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) feedback axis. These results demonstrate that GMSCs are capable of desensitizing allergic contact dermatitis via PGE<sub>2</sub>-dependent mechanisms. *STEM CELLS* 2011;29:1849–1860

Disclosure of potential conflicts of interest is found at the end of this article.

### INTRODUCTION

Mesenchymal stromal cells (MSCs), previously referred to as mesenchymal stem cells, possess multifunctional properties from tissue repair/regeneration to immunomodulatory functions [1–3]. An increasing body of evidence indicates that MSCs can home and engraft at the injured site and promote tissue repair through a combined downregulation of proinflammatory cytokines and increased production of growth factors, antioxidants, and soluble factors with anti-inflammatory functions [4, 5]. Studies have shown that MSCs exert their immunosuppressive functions via inhibiting the proliferation and activation of different subtypes of effector T cells, natural killer (NK) cells, dendritic cells (DCs), and macrophages or via promoting the differentiation of regulatory T cells (Tregs) [6–10]. These unique properties render MSC a potential novel immunotherapeutic tool for a variety of autoimmune and inflammation-related diseases [3, 4]. Recent studies have reported that MSCs can also suppress allergic responses and

chronic inflammation in experimental mouse models of ragweed- and ovalbumin-induced asthma [11, 12] and allergic rhinitis [13]. However, the underlying mechanism of MSC-mediated attenuation of allergic response still remains elusive.

The hapten-induced murine contact hypersensitivity (CHS) is an experimental model for human allergic contact dermatitis (ACD), one of the prevalent skin diseases worldwide with significant economic burden [14, 15]. Comparable to the pathophysiology of human ACD, the murine CHS model comprises three phases: the sensitization phase (also termed as the afferent or induction phase), the elicitation or challenge phase, and the resolution/regulation phase [14]. The sensitization phase is initiated immediately after the first exposure of skin to haptens, during which dermal DCs uptake and process antigens and then migrate to regional draining lymph nodes (dLNs), where they stimulate the differentiation and activation of allergen-specific T lymphocytes, including CD8<sup>+</sup> cytotoxic and CD4<sup>+</sup> T helper cells. In the elicitation phase, allergen-specific effector CD8<sup>+</sup> and CD4<sup>+</sup> T cells and various types of innate immune cells, particularly mast cells

Author contributions: W.-R.S. and Q.-Z.Z.: conception and design, collection and assembly of data, manuscript writing, and final approval of the manuscript; S.-H.S. and A.L.N.: collection and assembly of data; and A.D.L.: conception and design, manuscript writing, final approval of the manuscript, and financial support.

Correspondence: Anh D. Le, Division of Endodontics, Oral & Maxillofacial Surgery & Orthodontics, Center for Craniofacial Molecular Biology, The Herman Ostrow School of Dentistry of University of Southern California, Health Sciences Campus, 2,250 Alcazar Street, CSA 103, Los Angeles, California 90,033, USA. Telephone: 323-442-2556; Fax: 323-442-2981; e-mail: anhle@usc.edu Received June 6, 2011; accepted for publication September 4, 2011; first published online in *STEM CELLS EXPRESS* October 10, 2011. © AlphaMed Press 1066-5099/2009/\$30.00/0 doi: 10.1002/stem.738

(MCs), are activated and produce a plethora of inflammatory cytokines and mediators that contribute to the appearance of eczematous lesions. The resolution/regulation is characterized by the recruitment and activation of CD4<sup>+</sup> Tregs and other potential regulatory immune cells, which contribute to the resolution of inflammatory processes [14]. Currently, topical application of corticosteroid is the first-line palliative measure for ACD with short-term outcome, while allergen identification to improve contact avoidance is still challenging. Therefore, it would be promising to develop a more effective desensitizing tool based on specific cellular targets engaged by multiple types of innate and adaptive immune cells in the complex but distinctively phased pathophysiological processes of ACD. The unique immunomodulatory functions of MSCs on various types of immune cells may render them as a novel approach to desensitize allergic diseases.

We have recently isolated a unique subpopulation of MSCs from human gingival tissues, designated as GMSCs [16], with similar immunomodulatory properties to human bone marrow-derived MSCs (BMSCs), including the inhibition of T-cell proliferation and activation, enhancement of Treg generation and polarization of M2 macrophages [10, 16]. In the present study, we further explored the immunomodulatory functions of GMSCs in an established mouse model of delayed-type allergic responses or CHS and the potential cellular and molecular mechanisms.

## MATERIALS AND METHODS

### Animals

BALB/c mice (male, 8-10 weeks old) were obtained from Jackson Laboratories (Bar Harbor, ME, <http://www.jax.org>) and group-housed at the Animal Facility of University of Southern California (USC). All animal care and experiments were performed under institutional protocols approved by the Institutional Animal Care and Use Committee (IACUC) at USC.

### Antibodies and Reagents

Lipopolysaccharides (LPS), phorbol 12-myristate 13-acetate (PMA), A23187, indomethacin, oxazolone, and brefeldin A were from Sigma (St. Louis, MO, <http://www.sigmaaldrich.com>). Antibodies include anti-CD117-APC, anti-Fc $\epsilon$ RI $\alpha$ -FITC, anti-interleukin (IL)-6-PE, anti-IL-4-PE, anti-tumor necrosis factor (TNF)- $\alpha$ -PE anti-CD4-PerCP-Cy5.5, anti-CD25-PE, and anti-FoxP3-FITC (eBioscience, San Diego, CA, <http://www.ebioscience.com>).

### Cell Culture

Human gingival samples were collected from healthy subjects following routine dental procedures at USC School of Dentistry under the approved institutional review board. GMSCs were isolated and cultured as previously described [16].

CD14<sup>+</sup> cells were negatively selected from human peripheral blood mononuclear cells (PBMCs) (AllCells LLC, Emeryville, CA, <http://www.allcells.com>) using the MACS CD14 MicroBeads (Monocyte Isolation Kit, Miltenyi Biotec, Auburn, CA, <http://www.miltenyibiotec.com>) according to the manufacturer's instructions. Purity of isolated monocytes was assessed by flow cytometry. Cells were then cultured in six-well plates in RPMI 1,640 medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, 1% L-glutamine (Invitrogen, Carlsbad, CA, <http://www.invitrogen.com>), and recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF; 20 ng/ml) and IL-4 (20 ng/ml; PeproTech, Rocky Hill, NJ, <http://www.peprotech.com>).

The human MC line (HMC-1) was kindly provided by Dr. J.H. Butterfield (Mayo Clinic, Rochester, MN) [17]. Cells were main-

tained in Iscove's modified Dulbecco's medium supplemented with 10% FBS, 100 IU/ml penicillin, and 100  $\mu$ g/ml streptomycin.

Based on previous studies, the inhibitory effects of MSCs on DCs were observed in the range of 1:10-1:1 MSC/monocyte ratios with the maximum inhibitory effect reported at 1:1 ratio [8, 18, 19]. We therefore selected the 1:1 cell ratio of monocyte/DCs or MCs in all the coculture experiments under either direct cell-cell contact or transwell (TW) conditions for the mechanistic studies. Neutralizing Abs specific for human IL-10, transforming growth factor (TGF)- $\beta$ 1, TNF- $\alpha$ , or an isotype-matched monoclonal antibody (10  $\mu$ g/ml; R&D Systems, Minneapolis, MN, [www.RnDSystems.com](http://www.RnDSystems.com)), or indoleamine-2,3-dioxygenase (IDO) inhibitor (1-methyl-L-tryptophan [1-MT]; Sigma-Aldrich) were used for certain experimental purposes. Since endogenous PGE<sub>2</sub> produced by host cells may play an important role in CHS development [20, 21], then under certain conditions we chose to pretreat GMSCs with 5  $\mu$ M indomethacin (Sigma-Aldrich) in vitro to completely block the exogenous source of PGE<sub>2</sub> release and then injected into mice, wherein the inhibitory effects can be maintained for at least 1 week [22].

### Flow Cytometry

Cell surface markers and intracellular cytokine were analyzed using a FACS Calibur (BD Biosciences, San Jose, CA, <http://www.bdbiosciences.com>) following standard protocols.

### Treatment of CHS by GMSCs

The murine CHS model was induced as described previously [23]. Briefly, 20  $\mu$ l of a 1% oxazolone solution in acetone/sesame seed oil (4:1) was applied to the right ear. Seven days later, the sensitized right ears were challenged with 20  $\mu$ l of 1% oxazolone. An identical amount of acetone/sesame seed oil (4:1) was administered to the left ear as control. Based on the disease course of CHS, different treatment regimens were performed, whereby GMSCs prelabeled with CM-DiI (2.0  $\times$  10<sup>6</sup> per mice) were intravenously injected into mice either 1 day before sensitization (treatment regimen I), or 1 day before initiation or challenge (treatment regimen II), or 1 hour after challenge (treatment regimen III) (Fig. 1A). Control group received phosphate-buffered saline only or normal skin-derived fibroblasts (*n* = 4). Ear thickness was measured in a blind way at indicated time points. Mice were sacrificed on day 2 after challenge and ear samples were harvested for further analysis.

### Immunohistochemical and Western Blot Analysis

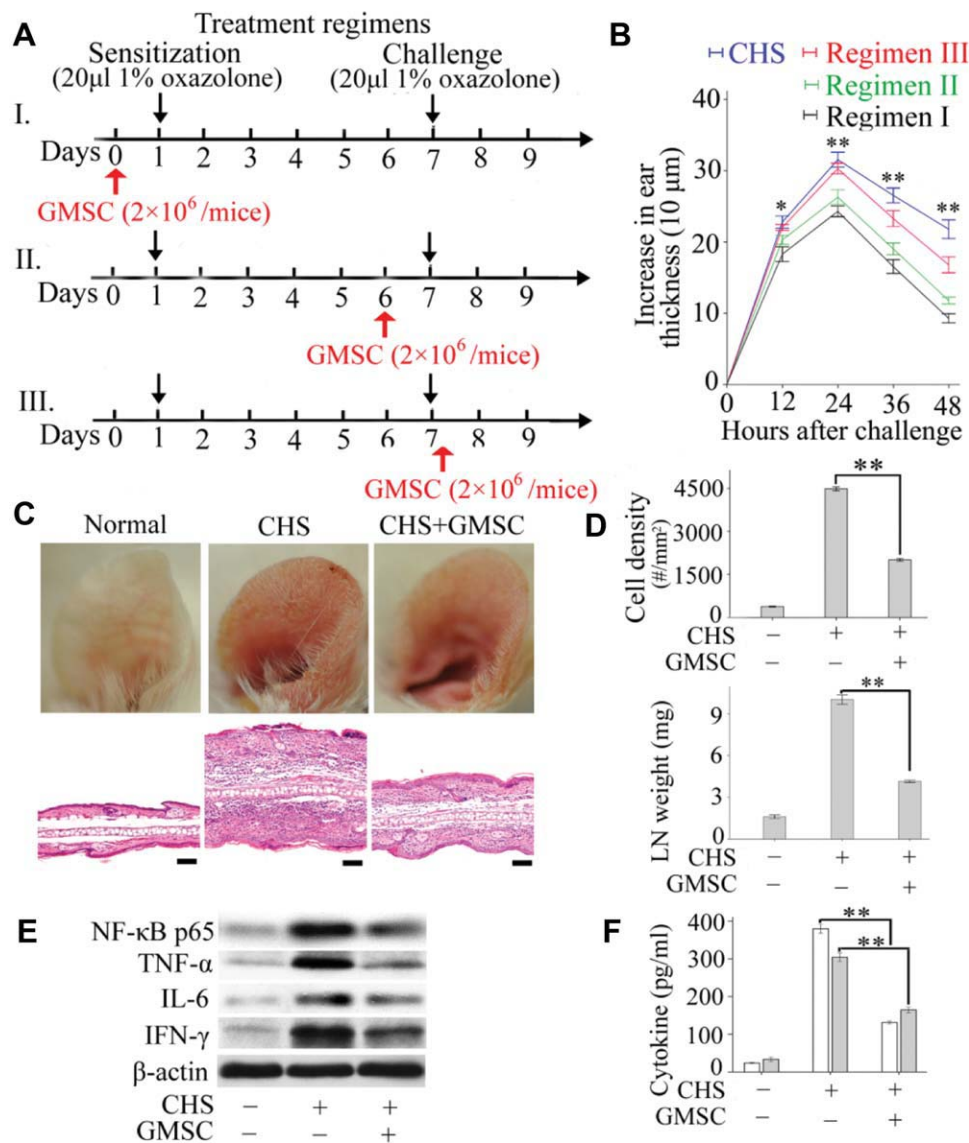
H&E and toluidine blue staining was performed on paraffin-embedded sections for histological and MC examination. Immunofluorescence studies were performed using specific antibody for mice Fc $\epsilon$ RI $\alpha$ . For semiquantification, positive signals in at least five random high-power fields were visualized and counted. Western blot analysis was performed as previously described [16], with antibodies specific for mice TNF- $\alpha$ , IL-6, IL-4, interferon-gamma (IFN- $\gamma$ ), IL-10 (Biolegend, San Diego, CA, <http://www.biolegend.com>), or human cyclooxygenase (COX)-2, nuclear factor-kappaB (NF- $\kappa$ B) p65 (Millipore, Billerica, MA) or  $\beta$ -actin (Sigma).

### Enzyme-Linked Immunosorbent Assay

The concentration of cytokines in ear lysates and the supernatants of cultured cells were detected using enzyme-linked immunosorbent assay (ELISA) kits (eBioscience), which was normalized according to the protein concentration and cell number, respectively. The production of PGE<sub>2</sub> in supernatants was determined using an EIA kit (Cayman Chemical).

### Statistical Analysis

All data are expressed as mean  $\pm$  SEM from at least three independent experiments, and statistical analyses were performed by means of appropriate one-way analysis of variance or the Student's *t* test using SPSS software (SPSS 16.0, Inc, Chicago, IL). Differences were considered statistically significant when a *p* value is less than .05.



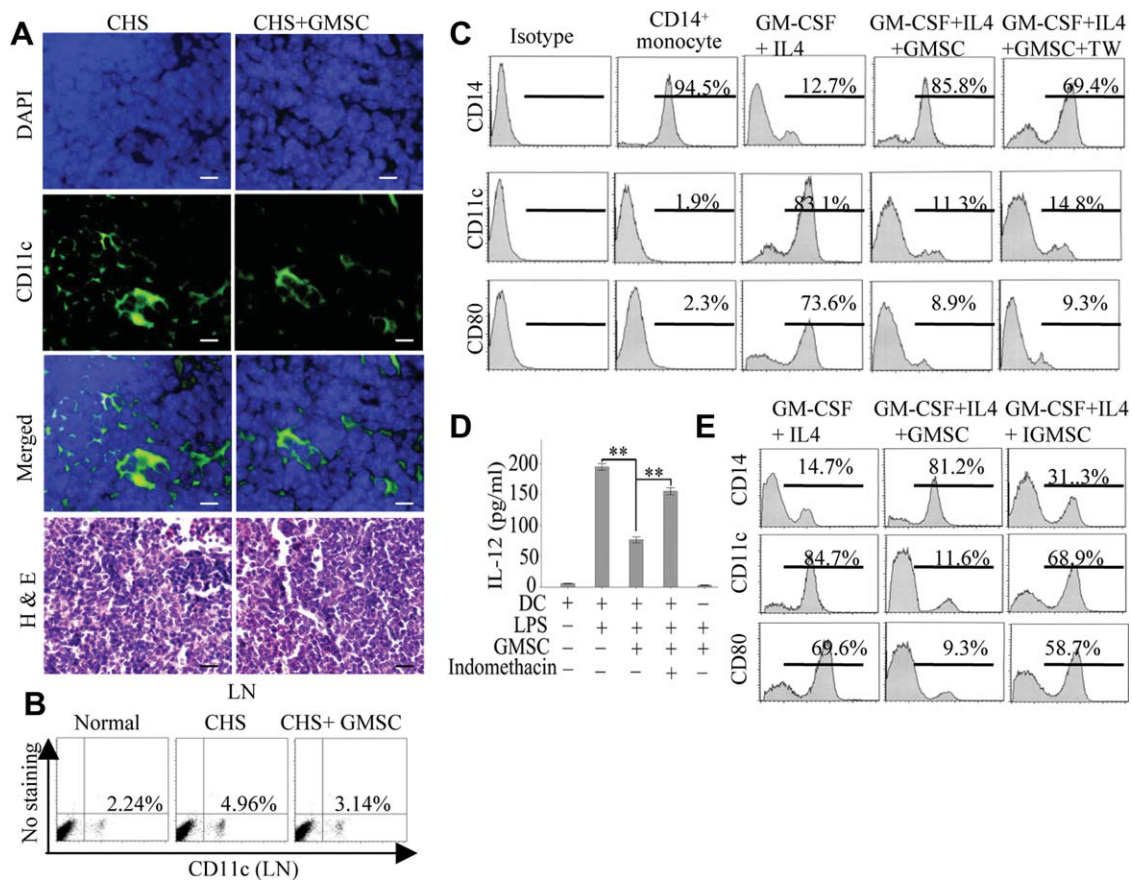
**Figure 1.** GMSC-based therapy attenuates CHS. (A): Experimental protocols showing different treatment regimens using GMSCs. GMSCs ( $2 \times 10^6$  per mice) were systemically injected into mice via tail vein either 1 day before sensitization with 1% oxazolone (regimen I), 1 day before challenge (regimen II), or 1 hour after challenge with 1% oxazolone (regimen III). Forty-eight hours after challenge, ear and local dLN samples were collected for further analysis. (B): Ear thickness ( $n = 4$ ) was measured at the indicated times after challenge in different experimental groups. (C): Representative photos of the ear and images of H&E staining of ear samples from mice following treatment regimen II with GMSCs. Scale bars, 200  $\mu$ m. (D): Quantification of cellular components in CHS ears and dLNs. (E, F): GMSCs inhibited the production of inflammatory cytokines in local ears as determined by Western blot and enzyme-linked immunosorbent assay. The results represent three independent experiments (mean  $\pm$  SEM). \* $p < .05$ ; \*\* $p < .01$  by one-way analysis of variance (B) and Student's  $t$  test (D, F). Abbreviations: CHS, contact hypersensitivity; GMSC, gingiva-derived mesenchymal stromal cell; IFN- $\gamma$ , interferon-gamma; IL-6, interleukin-6; LN, lymph node; NF- $\kappa$ B, nuclear factor-kappaB; and TNF- $\alpha$ , tumor necrosis factor-alpha.

## RESULTS

### Treatment with GMSCs Can Suppress CHS

According to the specific disease course of CHS, we first investigated the efficacy of GMSC treatment intervention at different stages of CHS (Fig. 1A). To this purpose, GMSCs ( $2 \times 10^6$  per mice) were systemically injected into mice either at 1 day before sensitization with 1% oxazolone (treatment regimen I), 1 day before challenge (treatment regimen II), or 1 hour after challenge (treatment regimen III) with 1% oxazolone, and the extent of swelling/inflammation in terms of external ear thickness was carefully measured at the inter-

vals of 12 hours. As shown in Figure 1B, even though GMSC treatment at different phases of CHS yielded variable therapeutic effects, systemic infusion of GMSCs following each of the three treatment regimens consistently led to statistically significant attenuation of CHS appearance when compared with untreated CHS mice from 24 to 48 hours after the first challenge (I or II vs. CHS control,  $p < .01$ ; III vs. CHS control,  $p < .05$ ). Of note, early intervention before antigen sensitization (treatment regimen I) exhibited the best efficacy, followed by treatment regimens II and III, respectively, characterized by a decrease in ear thickness and inflammation that appeared as early as 12 hours (I or II vs. III,  $p < .05$ ), and more apparently in the next 24-48 hours after the first challenge (I or II vs. III,  $p < .01$ ) (Fig. 1B, 1C). Even though the



**Figure 2.** Attenuation of CHS by GMSCs involves PGE<sub>2</sub>-dependent inhibition of DCs. (A): The infiltration of CD11c<sup>+</sup> DCs in the regional draining lymph nodes (dLNs) was determined by immunofluorescence staining. (B): The percentage of CD11c<sup>+</sup> DCs in dLNs was determined by flow cytometry. (C): GMSCs inhibited the differentiation and maturation of DCs. CD14<sup>+</sup> monocytes were cultured alone or cocultured with GMSCs (1:1) in direct cell-cell contact or in TWs in the DC-induction medium containing GM-CSF (20 ng/ml) and IL-4 (20 ng/ml). Seven days later, the number of CD11c<sup>+</sup> and CD80<sup>+</sup> DCs was determined by flow cytometry. (D): GMSCs dramatically reduced IL-12 secretion by DCs in response to LPS stimulation. (E): GMSCs pretreated with 5  $\mu$ M indomethacin (IGMSCs) were cocultured with CD14<sup>+</sup> monocytes in TWs under the same condition as described in C, and then the number of CD11c<sup>+</sup> and CD80<sup>+</sup> DCs was determined by flow cytometry. The results represent three independent experiments (mean  $\pm$  SEM). \* $p$  < .05; \*\* $p$  < .01 by Student's  $t$  test. Abbreviations: CHS, contact hypersensitivity; DAPI, DC, dendritic cell; GM-CSF, granulocyte-macrophage colony-stimulating factor; GMSC, gingiva-derived mesenchymal stromal cell; IGMSC, indomethacin-pretreated mesenchymal stromal cell; IL-4 and -12, interleukin-4 and -12; LN, lymph node; LPS, lipopolysaccharides; and TW, transwell.

therapeutic effect of treatment regimen I appeared more pronounced than that of treatment regimen II, the difference was not statistically significant (I vs. II,  $p$  > .05). In addition, treatment regimen II in combination with III appeared more effective in suppressing CHS than treatment regimen II alone; however, the difference is not statistically significant ( $p$  > .05) (Supporting Information Fig. S1A). Similar therapeutic effects were observed after treatment with bone marrow-derived MSCs 1 day before challenge (treatment regimen II), but treatment with skin fibroblasts showed no obvious therapeutic effects on CHS (Supporting Information Fig. S1B). Taken together, these findings suggest that systemic infusion of GMSCs before sensitization and challenge yielded optimal treatment effect on CHS. Therefore, we chose treatment regimen II in most of the following studies unless specifically indicated.

Further analysis of ear specimens and regional dLNs harvested 48 hours after challenge showed a markedly reduction in infiltration of inflammatory cells and gross LN weight in GMSC-treated mice as compared to untreated CHS controls (Fig. 1D). We next investigated the in vivo effects of GMSCs on the production of local inflammatory cytokines at the CHS sites. Results from both Western blot and ELISA showed a significant increase in the expression of NF- $\kappa$ B p65 and proinflammatory cytokines including TNF- $\alpha$ , IL-6, and IFN- $\gamma$  in tissue

lysates of CHS ears when compared with those of normal controls, whereas treatment with GMSCs significantly decreased the local expression of these inflammation-related genes (Fig. 1E, 1F). These findings suggest that GMSCs are capable to harness delayed-type hypersensitivity reactions by suppressing a variety of inflammatory cytokines and gene products.

### GMSCs Attenuate CHS via PGE<sub>2</sub>-Dependent Inhibition of DCs

Previous studies have demonstrated that DCs play a critical role in the initiation of CHS [24–27], while bone marrow- and adipose-derived MSCs are capable to inhibit their immune functions [8, 18, 19]. Here, we postulated that attenuation of CHS by GMSCs might involve the inhibition of DC functions. To test our hypothesis, GMSCs ( $2 \times 10^6$  per mice) were systemically injected into mice 1 day before sensitization and sacrificed 2 days after challenge. Immunostaining showed that GMSC treatment robustly reduced the number of CD11c<sup>+</sup> DCs at both the regional dLNs (Fig. 2A) and the antigen challenged ears (Supporting Information Fig. S1C). The reduction in the number of CD11c<sup>+</sup> DCs in LNs caused by GMSC treatment was further confirmed by flow cytometric analysis (Fig. 2B).

We next performed a series of *in vitro* studies to confirm the inhibitory effect of GMSCs on DCs. To this end, PBMC-derived CD14<sup>+</sup> monocytes were cocultured with GMSCs (1:1) under the condition of direct cell-cell contact or in a TW system in the DC-induction medium, whereas CD14<sup>+</sup> monocytes cultured alone under the same condition were served as controls. After 7 days, DC differentiation was assessed by flow cytometric analysis for expression of CD11c and the costimulatory molecule, CD80, both are cell surface molecules expressed by matured DCs. As shown in Figure 2C, GMSCs cocultured with CD14<sup>+</sup> monocytes under both cell-cell contact and TW conditions significantly inhibited the differentiation and maturation of DCs, characterized by a reciprocal increase in the expression of CD14 and a decrease in the expression of CD11c and CD80, when compared with CD14<sup>+</sup> monocytes-derived DCs cultured alone (Fig. 2C). Functionally, we found coculture with GMSCs dramatically reduced IL-12 secretion by DCs in response to LPS stimulation (Fig. 2D), which was further confirmed by flow cytometry after intracellular staining (Supporting Information Fig. S1D). These results suggest that soluble factors contribute to GMSC-mediated inhibitory effects on the differentiation and activation of DCs.

To further define specific secretory factors involved in GMSC-mediated inhibition of DCs, monoclonal neutralizing antibodies specific for TGF- $\beta$ 1 and IL-10 (10  $\mu$ g/ml), or specific inhibitor of IDO (1-MT, 500  $\mu$ M), or specific inhibitor of COX-1/2 (indomethacin, 5  $\mu$ M), were applied to the coculture system. We found that neutralizing TGF- $\beta$ 1 or IL-10 and pretreatment with 1-MT showed no obvious effects on GMSC-mediated inhibitory effects on DC differentiation (Supporting Information Fig. S1E, S1F) and LPS-stimulated IL-12 secretion by DCs (Supporting Information Fig. S1G). On the contrary, high levels of COX-2 expression and PGE<sub>2</sub> production were detected in GMSCs cocultured with CD14<sup>+</sup> monocytes under DC differentiation conditions (Supporting Information Fig. S1H, S1I), whereas pretreatment of GMSCs with 5  $\mu$ M indomethacin significantly but not completely reversed their inhibitory effect on DC differentiation (Fig. 2E) and LPS-stimulated IL-12 secretion by DCs (Fig. 2D; Supporting Information Fig. S1D, S1G); while this concentration of indomethacin was sufficient to completely abolish the increased PGE<sub>2</sub> production in GMSCs stimulated by cocultured CD14<sup>+</sup> monocytes without obvious cytotoxicity (Supporting Information Fig. S1H-S1J). These results suggest that GMSC-derived PGE<sub>2</sub> plays a crucial role in GMSC-mediated inhibitory effect on DCs.

Next, we asked whether PGE<sub>2</sub> is essential for GMSC-mediated desensitizing of CHS and the associated decrease in the number of DCs at the regional LNs. We first demonstrated that most GMSCs homed to the inflammatory sites still constitutively expressed COX-2 (Supporting Information Fig. S2A). Next, we pretreated GMSCs ( $2 \times 10^6$  per mice) with indomethacin and systemically injected them into mice 1 day before sensitization (treatment regimen I) or 1 day before challenge (treatment regimen II). Our results indicated that GMSCs pretreated with indomethacin lost their inhibitory capacity on DC infiltration in LNs collected following treatment regimen I (Supporting Information Fig. S2B); and more importantly, indomethacin treated GMSCs lost their capacity to attenuate CHS under both treatment regimens when compared with untreated GMSC (Supporting Information Fig. S2C, S2D). In addition, subcutaneous administration of 16,16-dimethyl PGE<sub>2</sub> (dmPGE<sub>2</sub>; 5-20  $\mu$ g/kg) led to a dose-dependent suppression of CHS appearance, but the treatment response was not as significant as that mediated by GMSC infusion (Supporting Information Fig. S2E). These compelling findings support the

notion that PGE<sub>2</sub> is indispensable to GMSC-mediated inhibition of DC functions both *in vitro* and *in vivo*, which might contribute, at least in part, to the underlying mechanism of GMSC-induced immunosuppression in CHS.

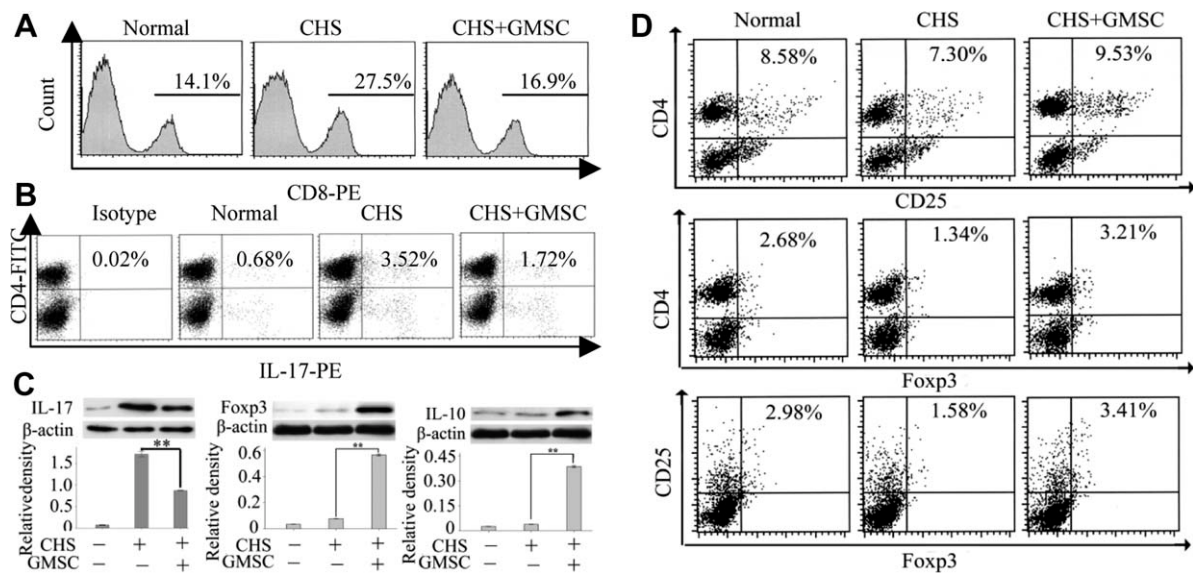
### Attenuation of CHS by GMSCs is Associated with Their Modulatory Effects on Multiple Effector T Cells

Besides DCs, multiple subtypes of T lymphocytes, including CD8<sup>+</sup> cytotoxic T cells [28, 29] and CD4<sup>+</sup> T helper cells, especially T<sub>H</sub>-17 [30–32] and Tregs [33, 34], partake different roles in the elicitation and resolution of CHS [14]. Here, we postulate that GMSC-mediated immunomodulatory effects on multiple types of T cells also contribute to their desensitizing therapeutic effects on CHS. As expected, systemic injection of GMSCs into mice 1 day before challenge (treatment regimen II) significantly reduced the infiltration of CD8<sup>+</sup> T cells at the regional dLNs and challenged CHS ears as demonstrated by immunostaining (Supporting Information Fig. S3A-S3C) and flow cytometric analysis (Fig. 3A). Specifically, we observed a reduction in the number of infiltrated T<sub>H</sub>-17 cells in the LNs and a parallel decreased expression of IL-17 in CHS ears after treatment with GMSCs, when compared with CHS controls (Fig. 3B, 3C). Conversely, when compared with CHS controls, GMSC treatment significantly increased not only the expression of FoxP3 (the specific transcriptional factor expressed by Tregs) and the anti-inflammatory cytokine IL-10 (a signature cytokine of Tregs) at the challenged ears but also the number of infiltrated Tregs at regional LNs as determined by Western blot, ELISA, and flow cytometry, respectively (Fig. 3C, 3D; Supporting Information Fig. S3D). Since our results have shown that PGE<sub>2</sub> is essential for GMSC-mediated attenuation of CHS and the associated inhibition of DC functions (Supporting Information Fig. S2), we ask whether PGE<sub>2</sub> plays a similar role in GMSC-mediated inhibition of effector T cells. As expected, GMSC-mediated inhibition of CD8<sup>+</sup> and T<sub>H</sub>-17 cell infiltration at the LNs was partially reversed when GMSCs were pretreated with indomethacin before systemic injection into mice (Supporting Information Fig. S3C, S3E).

### Attenuation of CHS by GMSCs is Associated with Their Inhibitory Effects on MCs

In addition to DCs and multiple subtypes of effector T cells, recent studies have implicated the critical role of MCs in delayed-type allergic reactions [35–43]. Thus, we further investigated whether GMSCs had any effects on MC functions and their potential role in GMSC-mediated attenuation of CHS. We first explored the interactions of homed GMSCs with host MCs at the inflammatory/challenged ears following systemic infusion of GMSCs pre-labeled with CM-DiI 1 day before challenge. Our results showed a more robust homing of labeled GMSCs to the challenged ear when compared with the opposite normal ear (Fig. 4A); the homed GMSCs were in close proximity with Fc $\epsilon$ R1 $\alpha$  positive MCs as shown by laser confocal microscopy (Fig. 4B). We next investigated the *in vivo* effects of GMSCs on the degranulation functions of MCs at the challenged ears. Toluidine blue staining showed that the number of MCs and the percentage of degranulated MCs dramatically decreased in GMSC-treated mice ears when compared with the untreated group (Fig. 4C, 4D), whereas such inhibitory effects on MCs were significantly attenuated when GMSCs were pretreated with indomethacin before systemic injection into mice (Fig. 4D). Altogether, these findings suggest that PGE<sub>2</sub> provides a functional link between GMSCs and MCs and potentially contributes to GMSC-mediated reversal of delayed-type hypersensitivity (DTH) in CHS model.



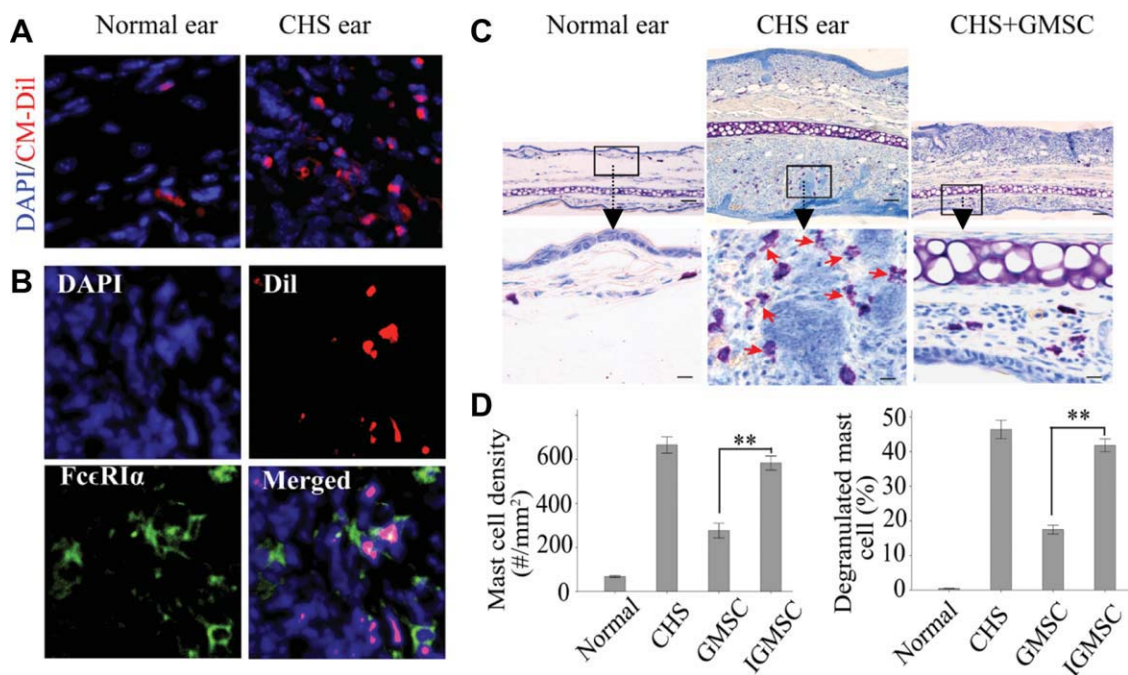


**Figure 3.** Attenuation of CHS by GMSCs is associated with their modulatory effects on multiple effector T cells. Following treatment regimen II with GMSCs, ear and local draining lymph node (dLN) samples were collected at 48 hours after challenge for further analysis. (A, B): The infiltration of CD8<sup>+</sup> T cells and T<sub>H</sub>-17 in the regional dLNs was determined by flow cytometry. (C): The expression of IL-17, Fc $\gamma$ 3, and IL-10 in CHS ears was determined by Western blot, wherein the graphs showed their relative density after normalization to the intensity of  $\beta$ -actin bands. (D): The infiltration of regulatory T cell in the regional dLNs was determined by flow cytometry. The results represent three independent experiments (mean  $\pm$  SEM). \* $p$  < .05; \*\* $p$  < .01 by Student's  $t$  test. Abbreviations: CHS, contact hypersensitivity; FITC, fluorescein isothiocyanate; Fc $\gamma$ 3, gamma3; GMSC, gingiva-derived mesenchymal stromal cell; IL-17, interleukin-17; IL-10, interleukin-10; and PE, phycoerythrin.

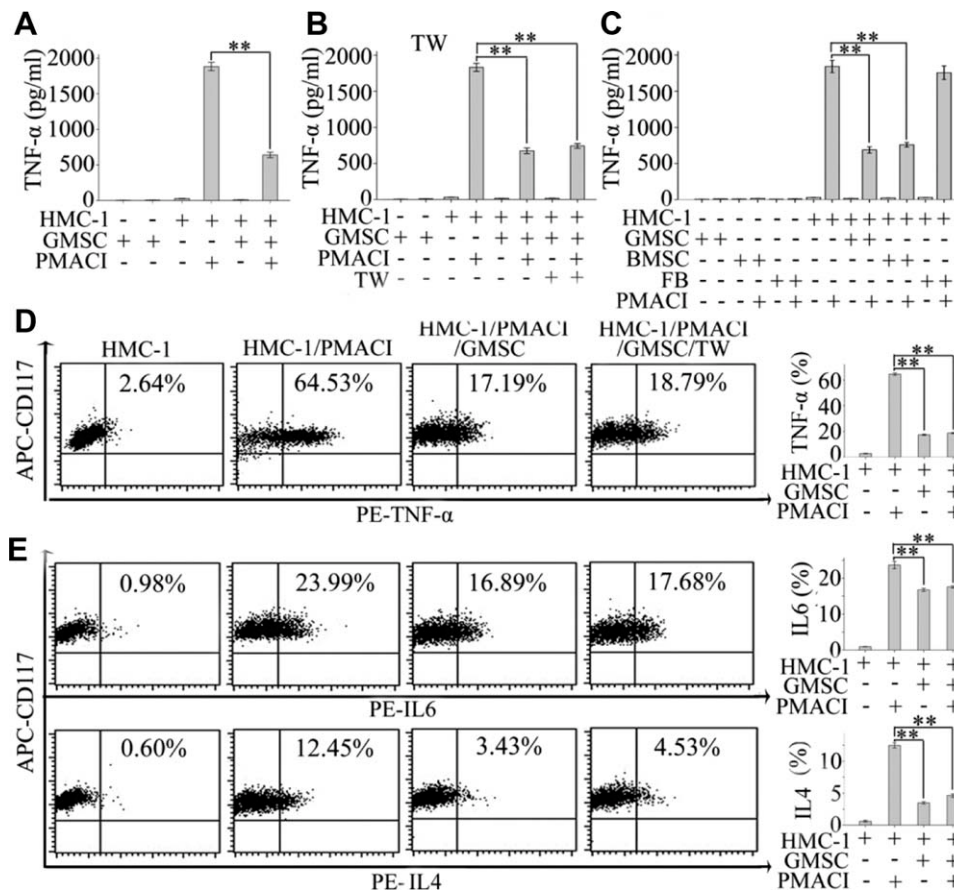
### Mechanisms Underlying GMSCs-Mediated Inhibition of MC Functions In Vitro

Next, we performed a series of in vitro studies to explore the interplay between GMSCs and MCs and their potential mech-

anisms. To this purpose, HMC-1 cells, an established human MC line, were cocultured with GMSCs at different ratios of cell density under direct cell-cell contact for 72 hours, followed by stimulation with PMA and calcium ionophore (PMACI) for another 18 hours. The production of TNF- $\alpha$  in



**Figure 4.** Interplay of homed GMSCs and mast cells (MCs) in CHS model. GMSCs prelabeled with CM-DiI were systemically injected into mice 1 day before challenge (regimen II). Forty-eight hours after challenge, ear samples were collected for further analysis. (A, B): GMSCs (red) homed to the inflammatory sites were in proximity with MCs (green) as determined by immunofluorescence staining with a specific antibody for Fc $\epsilon$ RI $\alpha$ . (C, D): GMSCs treatment decreased the number and degranulation of MCs as determined by toluidine blue staining. (red arrow: degranulated MCs). The results represent three independent experiments (mean  $\pm$  SEM). \* $p$  < .05; \*\* $p$  < .01 by Student's  $t$  test. Abbreviations: CHS, contact hypersensitivity; CM, CM-DiI; DAPI, 4',6-diamidino-2-phenylindole; DiI, 1,1'-diiodo-5,5'-dimethyl-3,3'-diphenylmethane; Fc $\epsilon$ RI $\alpha$ , Fc $\epsilon$ RI $\alpha$ ; GMSC, gingiva-derived mesenchymal stromal cell; and IGMSC, indomethacin-pretreated mesenchymal stromal cell.

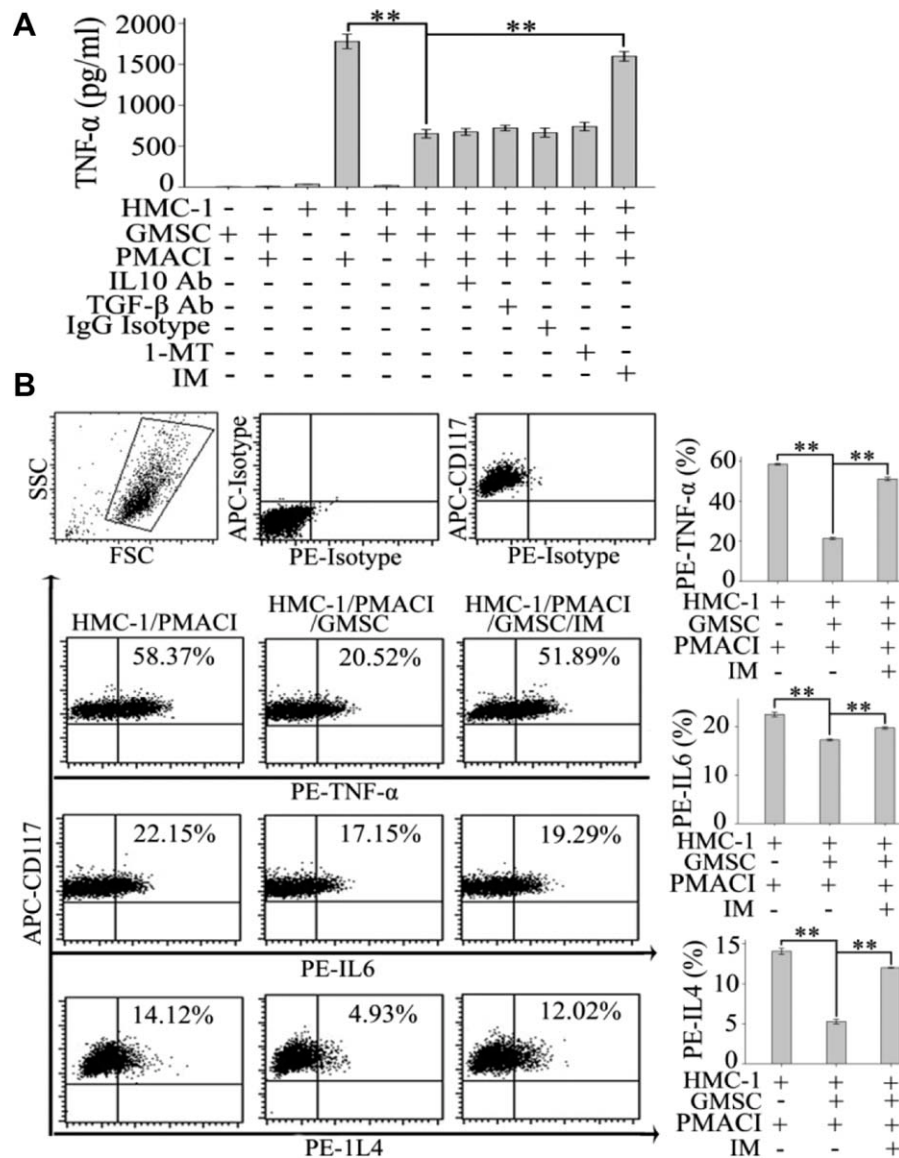


**Figure 5.** GMSCs suppress inflammatory cytokines release by HMC-1 cells. HMC-1 cells were cocultured with GMSCs (1:1) for 72 hours under direct cell-cell contact or in a TW system. (A, B) Following stimulation with PMACI, the secretory TNF- $\alpha$  in the supernatants were determined using enzyme-linked immunosorbent assay (ELISA). (C): HMC-1 cells were cocultured with BMSC or skin FBs (1:1) for 72 hours under in TWs. Following stimulation with PMACI, the secretory TNF- $\alpha$  in the supernatants were determined using ELISA. (D, E): The PMACI-stimulated expression of intracellular cytokines (TNF- $\alpha$ , IL-4, and IL-6) in HMC-1 after coculture with GMSCs in TWs were determined by flow cytometry. The results represent three independent experiments (mean  $\pm$  SEM). \* $p < .05$ ; \*\* $p < .01$  by Student's  $t$  test. Abbreviations: APC; BMSC, bone marrow-derived mesenchymal stromal cell; FB, fibroblast; GMSC, gingiva-derived mesenchymal stromal cell; HMC-1, human mast cell line; IL-4 and -6, interleukin-4 and -6; PMACI, phorbol 12-myristate 13-acetate and calcium ionophore; TNF- $\alpha$ , tumor necrosis factor-alpha; and TW, transwell.

the supernatants of MCs or GMSCs cultured alone and their cocultures was measured by ELISA. Our results showed that coculture with GMSCs under direct cell-cell contact led to a cell-dose-dependent inhibition of PMACI-stimulated TNF- $\alpha$  release by HMC-1 cells (Supporting Information Fig. S4A), with a maximum inhibition at equal cell density ratio (Supporting Information Fig. S4A; Fig. 5A;  $p < .01$ ). To determine whether inhibition of TNF- $\alpha$  release by GMSCs is dependent on direct cell-cell contact and/or soluble factors, HMC-1 cells and GMSCs were cocultured in the TW system. As shown in Figure 5B, coculture with GMSCs in TWs decreased PMACI-stimulated TNF- $\alpha$  release to a similar extent when compared with direct coculture, implying that soluble factors may play an essential role. However, coculture with an increasing number of GMSCs in TWs had no obvious effect on the proliferation of HMC-1 cells, therefore, ruled out the possibility that GMSC-mediated inhibition of MC activation was due to the inhibition of cell proliferation (Supporting Information Fig. S4B). Similar to above findings, a reproducible reduction of PMACI-stimulated TNF- $\alpha$  production in coculture with bone marrow-derived MSCs was observed ( $p < .01$ ), whereas no obvious changes were detected in cocultures with normal skin fibroblasts ( $p > .05$ ) (Fig. 5C). Sub-

stantially, the inhibitory effects of GMSCs on MC activation were further confirmed by flow cytometric analysis, which showed a significant decrease in the percentage of HMC-1 cells expressing TNF- $\alpha$ , IL-6, and IL-4 among total CD117<sup>+</sup> (c-kit) HMC-1 cells cocultured with GMSCs under both direct cell-cell contact and TW system following stimulation with PMACI ( $p < .01$ ) (Fig. 5D, 5E). Of note, PMACI-stimulated upregulation of NF- $\kappa$ B p65 was also abolished in HMC-1 cells when cocultured with GMSCs in TW when compared with HMC-1 cells cultured alone (Supporting Information Fig. S4C), which was apparently correlated with GMSC-mediated inhibition of TNF- $\alpha$  secretion by PMACI-activated HMC-1 cells. Taken together, these results suggest that GMSCs can potentially inhibit MC activation through secretory soluble factors.

We then aimed to identify the specific secretory soluble factors that potentially contributed to GMSC-mediated inhibition of proinflammatory cytokine synthesis by MCs. To this end, monoclonal neutralizing antibodies specific for TGF- $\beta$ 1 and IL-10 (10  $\mu$ g/ml), or a specific inhibitor for IDO (1-MT, 500  $\mu$ M), were added into the coculture. Our results indicated that blocking these factors failed to restore GMSC-mediated inhibition of TNF- $\alpha$  release by HMC-1 cells in response to PMACI stimulation (Fig. 6A). Our results from in vivo

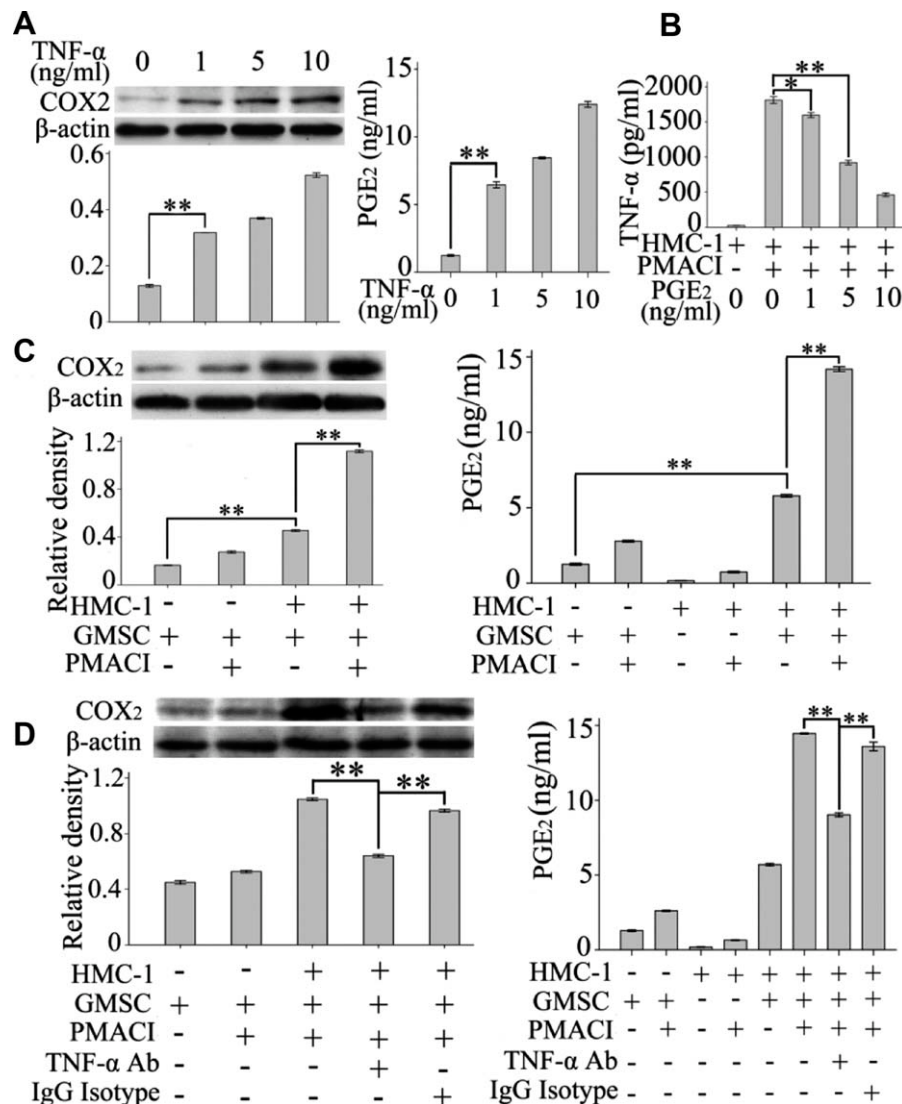


**Figure 6.** PGE<sub>2</sub> plays a key role in GMSC-mediated suppression of de novo synthesis of inflammatory cytokines in HMC-1 cells. HMC-1 cells were cocultured with GMSCs for 72 hours in transwells in the presence or absence of specific neutralizing antibodies (10 μg/ml) for either TGF-β1 or IL-10, or specific inhibitors for indomethacin (1-MT) or cyclooxygenase-2 (IM). (A): Following stimulation with PMACI, the secretory TNF-α in the supernatants was determined using enzyme-linked immunosorbent assay. (B): PMACI-stimulated expression of intracellular cytokines was determined using flow cytometry. The results represent three independent experiments (mean ± SEM). \**p* < .05; \*\**p* < .01 by Student's *t* test. Abbreviations: Ab; APC; FSC; GMSC, gingiva-derived mesenchymal stromal cell; HMC-1, human mast cell line; IL-4, -6, and -10, interleukin-4, -6, and -10; IM, indomethacin; 1-MT, 1-methyl-L-tryptophan; PE; PMACI, phorbol 12-myristate 13-acetate and calcium ionophore; SSC; TGF-β, transforming growth factor-beta; and TNF-α, tumor necrosis factor-alpha.

studies have assumed the potential role of PGE<sub>2</sub> in GMSC-mediated inhibition of MC functions during CHS (Fig. 4). To confirm this, we then pretreated GMSCs with indomethacin for 24 hours and then cocultured with HMC-1 cells in TWs followed by PMACI stimulation. We found that GMSCs pretreated with indomethacin lost their ability to inhibit PMACI-stimulated TNF-α release by HMC-1 cells (Fig. 6A). Flow cytometric analysis further demonstrated that pretreatment of GMSCs with indomethacin reversed their inhibitory effects on PMACI-activated HMC-1 cells in terms of TNF-α, IL-6 or IL-4 expression (Fig. 6B). These findings suggest that PGE<sub>2</sub> plays a critical role in GMSC-mediated inhibition of de novo synthesis of proinflammatory cytokines by MCs.

To further dissect the mechanisms whereby PGE<sub>2</sub> contributes to GMSC-mediated inhibition of MC activation, we first

exposed GMSCs to different concentrations of exogenous TNF-α for 24 hours, and the expression of COX-2 and production of PGE<sub>2</sub> were determined by Western blot and ELISA, respectively. Our results showed TNF-α treatment led to a dose-dependent increase in COX-2 expression and PGE<sub>2</sub> production by GMSCs (Fig. 7A, 7B). As expected, indomethacin-pretreated GMSCs failed to produce PGE<sub>2</sub> in response to stimulation with exogenous TNF-α (Supporting Information Fig. S4D). Conversely, treatment with exogenous PGE<sub>2</sub> inhibited PMACI-stimulated TNF-α secretion by HMC-1 cells in a dose-dependent manner (Fig. 7B). In addition, the expression of COX-2/PGE<sub>2</sub> was elevated in cocultured GMSCs and HMC-1 without PMACI stimulation, and such an increase was significantly augmented in the presence of PMACI stimulation (Fig. 7C). Likewise, the enhanced expression of



**Figure 7.** GMSCs interact with HMC-1 cells via TNF- $\alpha$ -PGE<sub>2</sub> feedback loop. (A, B): Exogenous TNF- $\alpha$  induces a dose-dependent increase in COX-2 expression and PGE<sub>2</sub> production in GMSCs. (B): Exogenous PGE<sub>2</sub> inhibits PMACI-induced TNF- $\alpha$  expression in a dose-dependent manner. (C): Cocultured HMC-1 cells in the presence of PMACI enhanced the COX-2 expression and the PGE<sub>2</sub> production by GMSCs. (D): Neutralizing TNF- $\alpha$  decreased COX-2 expression and PGE<sub>2</sub> production by GMSCs cocultured with HMC-1 cells stimulated by PMACI. The results represent three independent experiments (mean  $\pm$  SEM). \* $p$  < .05; \*\* $p$  < .01 by Student's  $t$  test. Abbreviations: Ab, COX2, cyclooxygenase2; GMSC, gingiva-derived mesenchymal stromal cell; HMC-1, human mast cell line; PGE<sub>2</sub>; PMACI, phorbol 12-myristate 13-acetate and calcium ionophore; and TNF- $\alpha$ , tumor necrosis factor-alpha.

COX-2/PGE<sub>2</sub> induced by PMACI-activated HMC-1 cells was abolished in the presence of TNF- $\alpha$  neutralizing antibody (Fig. 7D). Taken together, these results suggest that a critical feedback loop conferred by TNF- $\alpha$ /PGE<sub>2</sub> axis might play a key role in the crosstalks between MCs and GMSCs.

## DISCUSSION

ACD or CHS is one of the most common occupational diseases caused by repeated skin exposure to contact allergens such haptens [14, 15]. CHS belongs to type IV or DTH reactions, in which hapten-specific CD8<sup>+</sup> and CD4<sup>+</sup> effector or memory T cells secrete INF- $\gamma$ , IL-17, perforin, granzyme, and other inflammatory mediators, eliciting the efferent limb of the immune responses and amplifying the inflammatory reactions at the re-encounter of the same type of hapten [28–32].

Most recently, several lines of evidence have demonstrated the critical role of MCs in delayed-type allergic reactions, which contribute to CHS development by promoting the recruitment of neutrophils and DCs [38], by enhancing T-cell activation [37] as well as by regulating the magnitude and cytokine microenvironment of CHS response [44]. Meanwhile, emerging evidence also supports the important role of CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> T regulatory cells in preventing the development of allergic reactions to haptens/allergens contacting the skin, and in limiting the magnitude of the inflammatory process in already sensitized individuals [33, 34]. These studies have delineated the dynamics and complexity of cellular and molecular inflammatory networks contributory to the pathogenesis of CHS [14]. Therefore, simultaneously targeting multiple inflammatory signaling mediators and/or cellular components and reinducing tolerance to sensitizing haptens represent new modalities for the treatment of this type of allergic disease.

To date, numerous studies have well documented the immunomodulatory and anti-inflammatory properties of BMSCs and various other postnatal tissues [1–5] such as adipose [13, 18] and lung tissues [6], which have been used for the treatment of a variety of immune- and inflammation-related diseases [2–5]. In recent years, several studies have demonstrated that MSCs isolated from human dental tissues, including dental pulp [45, 46], dental follicle [46], apical papilla [47], periodontal ligament [48] and gingiva [16, 49], also exhibit similar *in vitro* immunosuppressive effects on T-cell functions when compared with BMSCs. Most recently, we and other groups have further reported some overlapping immunomodulatory functions of GMSCs and BMSCs on T cells and macrophages [10, 16, 49]. Herein, we further explored the therapeutic properties of GMSCs in a mouse model of CHS and found that GMSCs, similar to BMSCs, could ameliorate symptoms of CHS in mice (Supporting Information Fig. S1B). The attenuation of CHS induced by GMSC treatment was manifested as a significantly reduced infiltration of DCs, CD8<sup>+</sup> T cells and T<sub>H</sub>-17 effector cells at the regional draining LNs and local allergic areas, and a markedly increased infiltration of Tregs (Figs. 2, 3). More importantly, we found that treatment with GMSC also dramatically reduced the total number of MCs as well as the percentage of degranulated MCs in the allergic ears (Fig. 4). These findings suggest that GMSCs attenuate CHS through targeting multiple types of innate and adaptive immune cells, which constitute the cellular and molecular inflammatory cascade involved in the pathophysiology of CHS [14]. However, further studies are warranted to dissect the detailed mechanisms by which GMSCs affect the complex interactions among these immune cells during the CHS.

Even though the exact mechanism of MSC-mediated immunomodulatory functions *in vivo* remains largely unknown, a large number of *in vitro* studies have demonstrated the involvement of a variety of immunosuppressive factors, such as IL-10, TGF- $\beta$ 1, PGE<sub>2</sub>, nitric oxide (NO), and IDO, in MSC-mediated immunosuppression on various types of immune cells, including T lymphocytes, NK cells, DC, and macrophages [1–5, 7]. In the present study, we select to focus on DCs and MCs as major cellular players in GMSC-mediated immunosuppression pathways recognizing that DCs play critical role in the initiation of CHS [24–27] whereas MCs likely dominate CHS progression [35–44]. We showed that addition of specific neutralizing antibodies for IL-10 and TGF- $\beta$ 1 and a specific inhibitor for IDO failed to affect GMSC-mediated inhibition of DC and MC activation (Supporting Information Fig. S1E–S1G; Fig. 6A), but pretreatment of GMSCs with indomethacin, a specific inhibitor for COXs/PGE<sub>2</sub>, significantly reversed their inhibitory effects on DC function as well as the PMA-stimulated activation of MCs (Figs. 2, 6; Supporting Information Fig. S1E–S1G). Meanwhile, GMSCs pretreated with indomethacin before injection into mice lost their suppressive effects on CHS and correspondingly the infiltration and activation of DCs and MCs (Figs. 2, 4). These results are in agreement with previous findings that PGE<sub>2</sub> plays a key role in MSC-mediated suppressive effects on DCs [8, 18] and MCs [50]. On the contrary, to our best knowledge, evidence supporting the involvement of IL-10 and TGF- $\beta$ 1 in MSC-mediated suppression of DCs and MCs remains at large; a recent study reported that IDO knockout murine BMSCs could not suppress DC maturation and lacked immunomodulatory functions when compared with wild-type MSCs [51]. Taken together, these findings support the notion that PGE<sub>2</sub>, but not IL-10, TGF- $\beta$ 1, and IDO, plays a dominant role in GMSC-mediated attenuation of CHS and the underlying immunosuppressive effects on DCs and MCs.

As well characterized, PGE<sub>2</sub> exerts its biological functions via four subtypes of prostaglandin E receptors, EP1–EP4. The therapeutic effects of PGE<sub>2</sub> or its analogs on skin allergic inflammation have been described [52]; however, its efficacy and controversial results have been cautioned depending on the interaction with the receptor subtypes [21, 53–55]. For instance, PGE<sub>2</sub> suppressed skin allergic inflammation mediated via EP3 [21, 53] while triggering inflammatory response through Th1 cell and Th17 cell expansion via EP4 or EP1 [54, 55]. Such discrepancies may limit the clinical application of PGE<sub>2</sub> and its analogs in the treatment of skin allergic diseases. In the present study, we showed that subcutaneous administration of dmPGE<sub>2</sub> could partially ameliorate CHS in a dose-dependent manner; however, the suppressive effect is not as significant as that observed in animals treated with systemic infusion of GMSCs (Supporting Information Fig. S2E). Most importantly, MSCs, capable of both tropic and trophic activities, not only function to suppress the inflammatory and hypersensitivity phase of CHS but also contribute to the regeneration and repair of the injured tissues [56]. It has become increasingly apparent that even transient MSC engraftment may exert favorable effects through their trophic activities [4]. Most recently, we have demonstrated that labeled GMSCs could home to wounded skin sites and be detected even 1 week after systemic infusion [10]; this engraftment may provide additional lasting treatment benefit without the need for multiple dosing using a single pharmacological drug. All together, based on these unique properties, MSCs can function as a site-regulated multidrug dispensaries or “drugstore” [56] with multifunctional capacities, and therefore, are more superior to a single immunosuppressive or anti-inflammatory drug. Further studies should address the therapeutic cell dosage to optimize MSC application at both physiological and pharmacological levels.

In addition, we have evaluated the therapeutic effects of GMSCs on CHS using different treatment regimens and showed that systemic infusion of GMSCs following each of the three treatment regimens consistently attenuated CHS appearance in comparison with untreated CHS mice from 24 to 48 hours after challenge with the antigen, even if early intervention before antigen sensitization (treatment regimen I) and challenge (treatment regimen II) exhibited better efficacy than late intervention (treatment regimen III). This observation is consistent with previous reports in mice models of experimental autoimmune encephalomyelitis [57] and rheumatoid arthritis [58], whereby “prophylactic” administration of MSC, before disease onset, showed either similar or better ameliorative effects on the severity of the disease when compared with “therapeutic” administration of MSCs at early disease course, or at its peak [57, 58]. As reported, ACD is the most common occupational disorder; frequent recurrence and chronic cases have been reported in certain population of ACD patients due to continuous exposure to “unknown” allergens at the occupational settings [14, 15, 59]. Typically, ACD symptoms primed by the first encounter with allergens resolves in 7–21 days; but chronic ACD induced by continual exposure to allergens may persist for weeks to months even after antigen removal [15, 59]. Therefore, both prophylactic and therapeutic administrations of MSCs are necessary and particularly benefit chronic ACD patients in the allergen-exposed occupational settings. Further studies are warranted to explore the practical feasibility for the application of MSC-based therapy for the prevention and treatment of allergic diseases, including allergic rhinitis [13] and asthma [11, 60], knowingly that administration of MSCs during sensitization or before challenge also show significant ameliorative effects in mouse models.

## CONCLUSION

In summary, we have demonstrated that systemic application of GMSCs significantly suppressed both the sensitization and elicitation of CHS through modulating the function of multiple types of innate and adaptive immune cells through the COXs/PGE<sub>2</sub> pathway. These findings further support the notion that GMSCs, a unique population of MSCs with functional similarities to BMSCs, and specifically, their ease of isolation, accessible tissue source, and rapid *ex vivo* expansion, are a promising cell source for MSC-based therapies of inflammatory and allergic diseases.

## ACKNOWLEDGMENTS

This work was supported by National Institute of Health Research Grant, R01DE019932 and USC Institutional funding, CTSI and Zumberge awards. W.-R.S. and Q.-Z.Z. contributed equally to this work.

## DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

## REFERENCES

- Nauta AJ, Fibbe WE. Immunomodulatory properties of mesenchymal stromal cells. *Blood* 2007;110:3499–3506.
- Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. *Nat Rev Immunol* 2008;8:726–736.
- Zhao S, Wehner R, Bornhauser M et al. Immunomodulatory properties of mesenchymal stromal cells and their therapeutic consequences for immune-mediated disorders. *Stem Cells Dev* 2010;19:607–614.
- Tolar J, Le BK, Keating A et al. Concise review: Hitting the right spot with mesenchymal stromal cells. *Stem Cells* 2010;28:1446–1455.
- English K, French A, Wood KJ. Mesenchymal stromal cells: Facilitators of successful transplantation. *Cell Stem Cell* 2010;7:431–442.
- Jarvinen L, Badri L, Wettlaufer S et al. Lung resident mesenchymal stem cells isolated from human lung allografts inhibit T cell proliferation via a soluble mediator. *J Immunol* 2008;181:4389–4396.
- Kim J, Hematti P. Mesenchymal stem cell-educated macrophages: A novel type of alternatively activated macrophages. *Exp Hematol* 2009;37:1445–1453.
- Spaggiari GM, Abdelrazik H, Becchetti F et al. MSCs inhibit monocyte-derived DC maturation and function by selectively interfering with the generation of immature DCs: Central role of MSC-derived prostaglandin E2. *Blood* 2009;113:6576–6583.
- Spaggiari GM, Capobianco A, Abdelrazik H et al. Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: Role of indoleamine 2, 3-dioxygenase and prostaglandin E2. *Blood* 2008;111:1327–1333.
- Zhang QZ, Su WR, Shi SH et al. Human gingiva-derived mesenchymal stem cells elicit polarization of m2 macrophages and enhance cutaneous wound healing. *Stem Cells* 2010;28:1856–1868.
- Nemeth K, Keane-Myers A, Brown JM et al. Bone marrow stromal cells use TGF-beta to suppress allergic responses in a mouse model of ragweed-induced asthma. *Proc Natl Acad Sci USA* 2010;107:5652–5657.
- Bonfield TL, Kolozo M, Lennon DP et al. Human mesenchymal stem cells suppress chronic airway inflammation in the murine ovalbumin asthma model. *Am J Physiol Lung Cell Mol Physiol* 2010;299:L760–L770.
- Cho KS, Park HK, Park HY et al. IFATS collection: Immunomodulatory effects of adipose tissue-derived stem cells in an allergic rhinitis mouse model. *Stem Cells* 2009;27:259–265.
- Vocanson M, Hennino A, Rozieres A et al. Effector and regulatory mechanisms in allergic contact dermatitis. *Allergy* 2009;64:1699–1714.
- Fonacier LS, Dreskin SC, Leung DY. Allergic skin diseases. *J Allergy Clin Immunol* 2010;125:S138–S149.
- Zhang Q, Shi S, Liu Y et al. Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. *J Immunol* 2009;183:7787–7798.
- Butterfield JH, Weiler D, Dewald G et al. Establishment of an immature mast cell line from a patient with mast cell leukemia. *Leuk Res* 1988;12:345–355.
- Yañez R, Oviedo A, Aldea M et al. Prostaglandin E2 plays a key role in the immunosuppressive properties of adipose and bone marrow tissue-derived mesenchymal stromal cells. *Exp Cell Res* 2010;316:3109–3123.
- Djouad F, Charbonnier LM, Bouffi C et al. Mesenchymal stem cells inhibit the differentiation of dendritic cells through an interleukin-6-dependent mechanism. *Stem Cells* 2007;25:2025–2032.
- Soontrapa K, Honda T, Sakata D et al. Prostaglandin E2-prostaglandin E receptor subtype 4 (EP4) signaling mediates UV irradiation-induced systemic immunosuppression. *Proc Natl Acad Sci USA* 2011;108:6668–6673.
- Honda T, Matsuoka T, Ueta M et al. Prostaglandin E(2)-EP(3) signaling suppresses skin inflammation in murine contact hypersensitivity. *J Allergy Clin Immunol* 2009;124:809–818.
- Highfill SL, Kelly RM, O'shaughnessy MJ et al. Multipotent adult progenitor cells can suppress graft-versus-host disease via prostaglandin E2 synthesis and only if localized to sites of allopriming. *Blood* 2009;114:693–701.
- Roberts LK, Spangrude GJ, Daynes RA et al. Correlation between keratinocyte expression of Ia and the intensity and duration of contact hypersensitivity responses in mice. *J Immunol* 1985;135:2929–2936.
- Zaba LC, Krueger JG, Lowes MA. Resident and “inflammatory” dendritic cells in human skin. *J Invest Dermatol* 2009;129:302–308.
- Kumamoto Y, Denda-Nagai K, Aida S et al. MGL2 dermal dendritic cells are sufficient to initiate contact hypersensitivity in vivo. *PLoS One* 2009;4:e5619.
- Fukunaga A, Khaskhely NM, Sreevidya CS et al. Dermal dendritic cells, and not Langerhans cells, play an essential role in inducing an immune response. *J Immunol* 2008;180:3057–3064.
- Allan RS, Waithman J, Bedoui S et al. Migratory dendritic cells transfer antigen to a lymph node-resident dendritic cell population for efficient CTL priming. *Immunity* 2006;25:153–162.
- Vocanson M, Hennino A, Cluzel-Tailhardat M et al. CD8+ T cells are effector cells of contact dermatitis to common skin allergens in mice. *J Invest Dermatol* 2006;126:815–820.
- He D, Wu L, Kim HK et al. CD8+ IL-17-producing T cells are important in effector functions for the elicitation of contact hypersensitivity responses. *J Immunol* 2006;177:6852–6858.
- Zhao Y, Balato A, Fischelevich R et al. Th17/Tc17 infiltration and associated cytokine gene expression in elicitation phase of allergic contact dermatitis. *Br J Dermatol* 2009;161:1301–1306.
- Larsen JM, Bonefeld CM, Poulsen SS et al. IL-23 and T(H)17-mediated inflammation in human allergic contact dermatitis. *J Allergy Clin Immunol* 2009;123:486–492.
- Pennino D, Eyerich K, Scarponi C et al. IL-17 amplifies human contact hypersensitivity by licensing hapten nonspecific Th1 cells to kill autologous keratinocytes. *J Immunol* 2010;184:4880–4888.
- Ring S, Karakhanova S, Johnson T et al. Gap junctions between regulatory T cells and dendritic cells prevent sensitization of CD8(+) T cells. *J Allergy Clin Immunol* 2010;125:237–246.
- Ring S, Oliver SJ, Cronstein BN et al. CD4+CD25+ regulatory T cells suppress contact hypersensitivity reactions through a CD39, adenosine-dependent mechanism. *J Allergy Clin Immunol* 2009;123:1287–1296.
- Biedermann T, Kneilling M, Mailhammer R et al. Mast cells control neutrophil recruitment during T cell-mediated delayed-type hypersensitivity reactions through tumor necrosis factor and macrophage inflammatory protein 2. *J Exp Med* 2000;192:1441–1452.
- Kakurai M, Monteforte R, Suto H et al. Mast cell-derived tumor necrosis factor can promote nerve fiber elongation in the skin during contact hypersensitivity in mice. *Am J Pathol* 2006;169:1713–1721.
- Nakae S, Suto H, Kakurai M et al. Mast cells enhance T cell activation: Importance of mast cell-derived TNF. *Proc Natl Acad Sci USA* 2005;102:6467–6472.
- Suto H, Nakae S, Kakurai M et al. Mast cell-associated TNF promotes dendritic cell migration. *J Immunol* 2006;176:4102–4112.
- Wang HW, Tedla N, Lloyd AR et al. Mast cell activation and migration to lymph nodes during induction of an immune response in mice. *J Clin Invest* 1998;102:1617–1626.
- Bryce PJ, Miller ML, Miyajima I et al. Immune sensitization in the skin is enhanced by antigen-independent effects of IgE. *Immunity* 2004;20:381–392.
- Kerdel FA, Belsito DV, Scotto-Chinnici R et al. Mast cell participation during the elicitation of murine allergic contact hypersensitivity. *J Invest Dermatol* 1987;88:686–690.

- 42 Kobayashi M, Nunomura S, Gon Y et al. Abrogation of high-affinity IgE receptor-mediated mast cell activation at the effector phase prevents contact hypersensitivity to oxazolone. *J Invest Dermatol* 2010; 130:725–731.
- 43 Nishida K, Hasegawa A, Nakae S et al. Zinc transporter Znt5/Slc30a5 is required for the mast cell-mediated delayed-type allergic reaction but not the immediate-type reaction. *J Exp Med* 2009;206:1351–1364.
- 44 Norman MU, Hwang J, Hulliger S et al. Mast cells regulate the magnitude and the cytokine microenvironment of the contact hypersensitivity response. *Am J Pathol* 2008;172:1638–1649.
- 45 Pierdomenico L, Bonsi L, Calvitti M et al. Multipotent mesenchymal stem cells with immunosuppressive activity can be easily isolated from dental pulp. *Transplantation* 2005;80:836–842.
- 46 Tomic S, Djokic J, Vasilijic S et al. Immunomodulatory properties of mesenchymal stem cells derived from dental pulp and dental follicle are susceptible to activation by toll-like receptor agonists. *Stem Cells Dev* 2011;20:695–708.
- 47 Ding G, Wang W, Liu Y et al. Effect of cryopreservation on biological and immunological properties of stem cells from apical papilla. *J Cell Physiol* 2010;223:415–422.
- 48 Wada N, Menicanin D, Shi S et al. Immunomodulatory properties of human periodontal ligament stem cells. *J Cell Physiol* 2009;219: 667–676.
- 49 Tang L, Li N, Xie H et al. Characterization of mesenchymal stem cells from human normal and hyperplastic gingiva. *J Cell Physiol* 2011;226:832–842.
- 50 Brown JM, Nemeth K, Kushnir-Sukhov NM et al. Bone marrow stromal cells inhibit mast cell function via a COX2-dependent mechanism. *Clin Exp Allergy* 2011;41:526–534.
- 51 Ge W, Jiang J, Arp J et al. Regulatory T-cell generation and kidney allograft tolerance induced by mesenchymal stem cells associated with indoleamine 2,3-dioxygenase expression. *Transplantation* 2010; 90:1312–1320.
- 52 Rheins LA, Barnes L, Amornsiripanitch S et al. Suppression of the cutaneous immune response following topical application of the prostaglandin PGE<sub>2</sub>. *Cell Immunol* 1987;106:33–42.
- 53 Kunikata T, Yamane H, Segi E et al. Suppression of allergic inflammation by the prostaglandin E receptor subtype EP3. *Nat Immunol* 2005;6:524–531.
- 54 Yao C, Sakata D, Esaki Y et al. Prostaglandin E<sub>2</sub>-EP4 signaling promotes immune inflammation through Th1 cell differentiation and Th17 cell expansion. *Nat Med* 2009;15:633–640.
- 55 Nagamachi M, Sakata D, Kabashima K et al. Facilitation of Th1-mediated immune response by prostaglandin E receptor EP1. *J Exp Med* 2007;204:2865–2874.
- 56 Caplan AI, Correa D. The MSC: An injury drugstore. *Cell Stem Cell* 2011;9:11–15.
- 57 Zappia E, Casazza S, Pedemonte E et al. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. *Blood* 2005;106:1755–1761.
- 58 Bouffi C, Djouad F, Mathieu M et al. Multipotent mesenchymal stromal cells and rheumatoid arthritis: Risk or benefit? *Rheumatology (Oxford)* 2009;48:1185–1189.
- 59 Clark SC, Zirwas MJ. Management of occupational dermatitis. *Dermatol Clin* 2009;27:365–383.
- 60 Goodwin M, Sueblinvong V, Eisenhauer P et al. Bone marrow-derived mesenchymal stromal cells inhibit th2-mediated allergic airways inflammation in mice. *Stem Cells* 2011;29:1137–1148.



See [www.StemCells.com](http://www.StemCells.com) for supporting information available online.