An in Vitro and in Vivo Study of the Effect of Dexamethasone on Immunoinhibitory Function of Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells

Dan Wang¹, Yue-Qi Sun¹, Wen-Xiang Gao¹, Xing-Liang Fan¹, Jian-Bo Shi¹, and Qing-Ling Fu¹

Abstract
Induced pluripotent stem cell-derived mesenchymal stem cells (iPSC-MSCs) represent a promising cell source for patient-specific cell therapy. We previously demonstrated that they display an immunomodulatory effect on allergic airway inflammation. Glucocorticoids are powerful anti-inflammatory compounds and widely used in the therapy of allergic diseases. However, the effect of glucocorticoids on the immunomodulatory function of iPSC-MSCs remains unknown. This study aimed to determine the effect of dexamethasone (Dex) on the immunomodulatory function of iPSC-MSCs in vitro and in vivo. A total of three human iPSC-MSC clones were generated from amniocyte-derived iPSCs. Anti-CD3/CD28-induced peripheral blood mononuclear cell (PBMC) proliferation was used to assess the effect of Dex on the immunoinhibitory function of iPSC-MSCs in vitro. Mouse models of contact hypersensitivity (CHS) and allergic airway inflammation were induced, and the levels of inflammation in mice were analyzed with the treatments of iPSC-MSCs and Dex, alone and combined. The results showed that Dex did not interfere with the immunoinhibitory effect of iPSC-MSCs on PBMC proliferation. In CHS mice, simultaneous treatment with Dex did not affect the effect of iPSC-MSCs on the inflammation, both in regional draining lymph nodes and in inflamed ear tissue. In addition, co-administration of iPSC-MSCs with Dex decreased the local expression of interferon (IFN)-γ and tumor necrosis factor (TNF)-α in the ears of CHS mice. In the mouse model of allergic airway inflammation, iPSC-MSC treatment combined with Dex resulted in a similar extent of reduction in pulmonary inflammation as iPSC-MSCs or Dex treatment alone. In conclusion, Dex does not significantly affect the immunomodulatory function of iPSC-MSCs both in vitro and in vivo. These findings may have implications when iPSC-MSCs and glucocorticoids are co-administered.

Keywords
Induced pluripotent stem cell-derived mesenchymal stem cells (iPSC-MSCs), dexamethasone (Dex), immunoinhibitory, contact hypersensitivity (CHS), allergic airway inflammation

Introduction
Mesenchymal stem cells (MSCs) are multipotent cells with immunomodulatory functions on immune cells¹, which have been being clinically explored as a new therapeutic treatment for various immune-related diseases, including graft-versus-host disease (GvHD)², systemic lupus erythematosus (SLE)³, and Crohn’s disease⁴. Most preclinical and clinical studies are performed using MSCs derived from adult bone marrow (BM) and adipose tissue⁵-8. However, several disadvantages may restrict these adult MSCs for clinical applications, including the requirement of invasive techniques for...
their isolation, a limited number of cells obtained initially from a single donor, limited capacity to proliferate when cultured in vitro, and varying cell qualities from different donors. In the last decade, several reprogramming techniques generating human pluripotent stem cells (iPSCs) from adult somatic cells were successfully developed. These iPSCs hold enormous promise for patient-personalized cell therapies and for research into various human diseases, and importantly, represent an important alternative source of functional MSCs. Recently, we and others have successfully induced MSCs from iPSCs (iPSC-MSCs), which are similar to BM-MSCs in terms of morphology, multipotent differentiation potential, and expression of common MSC surface markers. The iPSC-MSCs display a strong immunomodulatory effect on natural killer (NK) cells, dendritic cells and T-cells. The induced iPSC-MSCs are potent in the protection of limb ischemia and doxorubicin-induced cardiomyopathy. We have previously demonstrated that, similar to BM-MSCs, iPSC-MSCs can inhibit the Th2 phenotype both in vitro and in vivo. Given the advantages of iPSC-MSCs over adult MSCs, there are large expectations for the use of iPSC-MSCs for the clinical application of the treatment of allergic diseases. MSCs in combination with glucocorticoids may provide further potential benefits in the treatment of several disorders, such as ulcerative colitis, kidney transplantation, and multiple sclerosis. Therefore, it is foreseen that iPSC-MSCs may be used for clinical purposes in combination with glucocorticoids, which is currently the gold standard for controlling inflammation in allergic diseases. Then, the effects of glucocorticoids on the function of iPSC-MSCs should be well studied in the case of combined administration.

Few studies have investigated the interaction between BM-MSCs and glucocorticoids. Human BM-MSCs have been reported to inhibit allogeneic lymphocyte proliferation in the presence of dexamethasone (Dex) in vitro. Conversely, a recent study demonstrated that Dex can eliminate the immunomodulatory effect of BM-MSCs on lymphocyte proliferation in vitro and in a murine model of liver fibrosis. In addition, a clinical trial using combined therapy with BM-MSCs and glucocorticoids in GvHD patients performed no better overall than a placebo. However, whether glucocorticoids interrupt the immunomodulatory function of iPSC-MSCs remains unknown.

In this study, we investigated the effect of Dex on the immunosuppression of iPSC-MSCs on the proliferation of anti-CD3/CD28-activated human peripheral blood mononuclear cells (PBMCs) in vitro, and examined the interactions between Dex and iPSC-MSCs in mouse models of contact hypersensitivity (CHS) and allergic airway inflammation. We found that Dex (at tested concentrations) had no significant effects on the immunomodulatory function of iPSC-MSCs, both in vitro and in vivo. It may be important for the potential clinical use of iPSC-MSCs in combination with glucocorticoids.

**Materials and Methods**

**Generation of Human iPSC-MSCs**

Amniocyte-derived iPSCs were purchased from Cell Inspire Bio (IPSN-0008, Shenzhen, China). Prior to generating the iPSC-MSCs, iPSCs were plated into a Matrigel-coated six-well plate, and cultured in mTeSR1 basal medium containing 20% mTeSR1 supplement (Stem Cell, MA, USA), 1% penicillin/streptomycin (Gibco, CA, USA), and sodium pyruvate, 10 mM 1-ascorbate-2-phosphate (Sigma, MO, USA) and changed every other day. At 2 weeks later, cells were split with a 1:2 ratio using 0.25% trypsin-ethylenediaminetetraacetic acid (Gibco, CA, USA), plated in a gelatin-coated six-well plate as passage one (P1). After two passages, cells were seeded in an uncoated six-well plate and cultured in iPSC-MSCs growth medium containing high glucose Dulbecco’s modified eagle’s medium (dMEM), 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, 200 mM l-glutamine and 10 mM non-essential amino acids (Gibco, CA, USA), sodium pyruvate, 10 mM 1-ascorbate-2-phosphate (Sigma, MO, USA) and changed every other day. The induced iPSC-MSCs are similar to BM-MSCs in terms of morphology, multipo
tent differentiation potential, and expression of common MSC surface markers. The iPSC-MSCs display a strong immunomodulatory effect on natural killer (NK) cells, dendritic cells and T-cells. The induced iPSC-MSCs are potent in the protection of limb ischemia and doxorubicin-induced cardiomyopathy. We have previously demonstrated that, similar to BM-MSCs, iPSC-MSCs can inhibit the Th2 phenotype both in vitro and in vivo. Given the advantages of iPSC-MSCs over adult MSCs, there are large expectations for the use of iPSC-MSCs for the clinical application of the treatment of allergic diseases. MSCs in combination with glucocorticoids may provide further potential benefits in the treatment of several disorders, such as ulcerative colitis, kidney transplantation, and multiple sclerosis. Therefore, it is foreseen that iPSC-MSCs may be used for clinical purposes in combination with glucocorticoids, which is currently the gold standard for controlling inflammation in allergic diseases. Then, the effects of glucocorticoids on the function of iPSC-MSCs should be well studied in the case of combined administration.

Few studies have investigated the interaction between BM-MSCs and glucocorticoids. Human BM-MSCs have been reported to inhibit allogeneic lymphocyte proliferation in the presence of dexamethasone (Dex) in vitro. Conversely, a recent study demonstrated that Dex can eliminate the immunomodulatory effect of BM-MSCs on lymphocyte proliferation in vitro and in a murine model of liver fibrosis. In addition, a clinical trial using combined therapy with BM-MSCs and glucocorticoids in GvHD patients performed no better overall than a placebo. However, whether glucocorticoids interrupt the immunomodulatory function of iPSC-MSCs remains unknown.

In this study, we investigated the effect of Dex on the immunosuppression of iPSC-MSCs on the proliferation of anti-CD3/CD28-activated human peripheral blood mononuclear cells (PBMCs) in vitro, and examined the interactions between Dex and iPSC-MSCs in mouse models of contact hypersensitivity (CHS) and allergic airway inflammation. We found that Dex (at tested concentrations) had no significant effects on the immunomodulatory function of iPSC-MSCs, both in vitro and in vivo. It may be important for the potential clinical use of iPSC-MSCs in combination with glucocorticoids.

**Differentiation of Human iPSC-MSCs**

Functional differentiation of iPSC-MSCs, including adipogenesis, osteogenesis and chondrogenesis, were carried out at passage 9. For adipocytic differentiation, cells were incubated in growth medium in six-well plates and changed to adipogenic differentiation medium (medium A for 3 days and medium B for 1 day; Cyagen Biosciences, CA, USA) when confluence was reached. After six cycles, cells were cultured in medium B for an additional 7 days, then stained with Oil Red O working solution (Cyagen Biosciences, CA, USA) and visualized under a light microscope. For osteogenic differentiation, cells were re-plated in growth medium at 3×10^4 cells/cm^2 in six-well plates pre-coated with gelatin solution. After 24 hours, the medium was changed to osteogenic differentiation medium (Cyagen Biosciences, CA, USA). After 3 weeks in culture, cells were fixed and stained with Alizarin red. For chondrogenic differentiation, 2.5×10^5 iPSC-MSCs were transferred to a 15-ml centrifugation tube to wash twice with incomplete chondrogenic medium (Cyagen Biosciences, CA, USA), then resuspended in complete chondrogenic medium to a density of 5.0×10^5 cells per ml. Cell suspension was transferred into a 15-ml polypropylene culture tube and centrifuged at 150×g for 5 minutes. The tube was incubated at 5% CO_2, 37°C, avoiding aspirating the supernatant or resuspending the pellet. After 24 hours, cell pellets were fed with fresh complete chondrogenic medium every 2–3 days.
Chondrogenic pellets were harvested after 28 days in culture, formalin fixed and paraffin embedded for Alcian blue stain.

**PBMC Proliferation Assay**

The buffy coats from anonymous healthy donors provided by Guangzhou Blood Center were used for human PBMC collecting as described previously\(^2^0\). The study protocol was approved by the Ethics Committee of the First Affiliated Hospital, Sun Yat-sen University, China (No. 2014-C-053), and exemption of written informed consent for using human buffy coats was approved. Cells were suspended in 500 μL of phosphate-buffered saline (PBS) containing 10% FBS and stained by 2 mM carboxyfluorescein succinimidyl amino ester (CFSE; Sigma, MO, USA). After 10 minutes, cells were washed twice with 10 mL RPMI 1640 medium (Hyclone, UT, USA) with 10% FBS, 1% penicillin/streptomycin, and 1% L-glutamine. Cells were resuspended and dispensed in 24-well plates at a density of 2 × 10⁵ cells/well. Then PBMCs with a stimulation of 1 μg/mL anti-CD3 and 1 μg/mL anti-CD28 (BD Biosciences, NJ, USA) were cultured alone or co-cultured with iPSC-MSCs in a ratio of 10:1, which was determined in our previous study\(^2^0\), in the absence or presence of Dex at concentrations ranging from 10 ng/mL to 100 μg/mL for 3 days.

**Flow Cytometry of PBMCs and iPSC-MSCs**

CFSE-stained PBMCs were harvested after 3 days of co-culture with iPSC-MSCs or Dex, and then the PBMC proliferation was assessed by flow cytometry (Beckman Gallios, IN, USA) using standard techniques. Cell surface antigens and human indoleamine 2,3-dioxygenase (IDO) expression in human iPSC-MSCs (passage 9) were also analyzed by flow cytometry. Antibodies against human antigens CD166, CD146, CD34, CD44, CD45, CD73, CD90, CD105 were purchased from BD Bioscience. Antibody against IDO (# P14902) was purchased from R&D systems (MN, USA). Data were analyzed by Kaluza Analysis Software (Beckman Coulter Life Sciences, IN, USA).

**Enzyme-linked Immunosorbent Assay**

Interleukin (IL)-6 and IL-10 levels in serum were determined using the ELISA Kit (KeyGEN BioTECH, Shanghai, China).

**Animals**

Female BALB/c mice (6–8 weeks) were purchased from Experimental Animal Center, Sun Yat-sen University (Guangzhou, China) and housed under specific pathogen-free conditions, maintained on a 12 h light/dark cycle, and provided food and water ad libitum.

All procedures were performed according to protocols approved by the Institutional Animal Care and Use Committee, Sun Yat-sen University.

**Mouse Contact Hypersensitivity Model**

Mice were sensitized to oxazolone (Sigma, MO, USA) by the application of 20 μL of 1% oxazolone in an acetone/ sesame seed oil vehicle (4:1 v/v) to both ears on day 1 and day 7\(^3^5\). iPSC-MSCs (1 × 10⁶ per mice, intravenous injection) or with Dex (5 mg/kg, intraperitoneal injection) were injected into mice at the same time on day 6. Control mice received PBS. Ears and draining auricular lymph nodes at the base of the ear were photographed on day 8 and 9 respectively and harvested on day 9. The biggest lymph node was weighed immediately after excision. Serum samples were collected on day 9 and serum IL-6 and IL-10 levels were determined by ELISA assay.

**Mouse Model of Allergic Airway Inflammation**

Ovalbumin (OVA)-induced mouse model of allergic airway inflammation was established as our previous study\(^2^1\). Briefly, mice were sensitized by intraperitoneal injection of 40 μg of OVA (grade V; Sigma, MO, USA) and 4 mg of aluminum hydroxide (Sigma, MO, USA) in 200 μL PBS on day 1 and day 14. From day 21 to 24, mice were challenged daily with aerosolized 5% OVA in a plexiglass chamber and through an air-compressing nebulizer for 30 minutes. iPSC-MSCs (1 × 10⁶ per mice) was injected via the tail vein on day 20. Mice were injected intraperitoneally with Dex (1.25 mg/kg/day) from day 21 to 24. The animals were sacrificed after the last challenge on day 24.

**Histology and Microscopic Measurement of Ear Thickness**

Ear biopsy specimens were fixed with 4% paraformaldehyde and embedded in paraffin. Deparaffinized 5-μm sections were stained with hematoxylin and eosin (H&E) and analyzed by Leica DM2500 microscope and Leica Application Suite (Leica, IL, USA) software. Ear thickness (including epidermis, dermis and subcutaneous layer) was determined by taking measurements on six individual sections.

**Evaluation of the Inflammatory Cells in the Bronchoalveolar Lavage Fluids**

On day 24, bronchoalveolar lavage fluids (BALFs) were collected with 1 mL of cold PBS via a 22-gauge needle inserted into the trachea. After centrifugation, cells in the BALFs were counted using a hemocytometer and then resuspended in 5 μL of PBS. The cell suspension was streaked in a thin film over a glass slide using the second slide as a spreader and was stained with Diff-Quik kit (D030, Nanjing Jiancheng Bioengineering Institute, China, http://www.njicbio.com). A total
of 250 cells per slide were evaluated for inflammatory cell counting.

**Lung Histology and Inflammation Scoring**

Lung sections were prepared at a thickness of 4-μm and were stained with H&E. For the lung inflammation quantification, five sections across the main bronchus and vascular per mouse were randomly selected. The inflammation levels were scored from 0 to 3 according to the following criteria: 0 = no inflammation; 1 = occasional inflammatory cells; 2 = most bronchus or vascular were surrounded by 1–5 layers of inflammatory cells; 3 = most bronchus or vascular were surrounded by more than five layers of inflammatory cells.

**Western Blotting**

Western blotting analysis of the homogenized mouse ears was performed following standard procedures. Antibodies against TNF-α (Cell Signaling Technology, MA, USA) and IFN-γ (Abcam, Cambridge, UK) were used.

**Statistical Analysis**

All data shown are expressed as means ± SEMs. Data were evaluated by one-way analysis of variance and further evaluated with the Bonferroni test for multiple comparisons using GraphPad Prism Software (CA, USA). A Student’s *t* test was also used to determine significance when applicable. *P*-values lower than 0.05 were considered to be significant.

**Results**

**Characterization of iPSC-MSCs**

Using a modification of previously described protocols, three human iPSC-MSC clones were successfully generated from iPSCs reprogrammed from amniocytes. The iPSC-MSCs displayed a fibroblastic morphology, which was distinct from their parent iPSCs but similar to BM-MSCs (Fig. 1(a)). Flow cytometry analysis showed that iPSC-MSCs expressed surface markers typical of adult MSCs, including CD105, CD73, CD90, CD166, CD146 and CD44, but did not express CD34 and CD45 (Fig. 1(b)). Trilineage differentiation assays demonstrated that iPSC-MSCs had the ability to differentiate along the chondrogenic, osteoblastic or adipocytic pathways when cultured in the appropriate conditions (Fig. 1(c)).

**Dex had no Effects on the Immunoinhibitory of iPSC-MSCs on PBMC Proliferation**

Previously, we have demonstrated that iPSC-MSCs have a potent ability to inhibit lymphocytes proliferation. However, whether Dex affects this ability remains unclear.

Therefore, we employed a co-culture system of lymphocyte proliferation induced by anti-CD3/CD28 stimulation to examine the effect of Dex on the immunoinhibitory function of iPSC-MSCs. PBMCs were separated from healthy volunteers’ blood samples and stimulated with anti-CD3/CD28 in the absence or presence of iPSC-MSCs and Dex. Consistent with our previous study, iPSC-MSCs alone significantly inhibited PBMC proliferation at a ratio of 1:10 (Fig. 2(a), upper panel, and Fig. 2(b), *P* < 0.001). As expected, Dex alone showed a dose-dependent inhibition of PBMCs proliferation (Fig. 2(a), lower panel, and Fig. 2(c), *P* < 0.01 or 0.001). After iPSC-MSCs were added with Dex with a graded concentration from 10 ng/ml to 100 μg/ml, the inhibition of PBMC proliferation was almost invariably similar to those when iPSC-MSCs were added alone (Fig. 2(a), upper and middle panel, and Fig. 2(d), *P* > 0.05), suggesting Dex does not affect the immunoinhibitory effects of iPSC-MSCs on PBMC proliferation.

Additionally, we analyzed whether IDO was involved in the inhibitory effects of iPSC-MSCs on PBMC proliferation. However, we did not observe any difference in IDO expression in iPSC-MSCs after co-culture with PBMCs in the presence or absence of Dex (Supplementary Fig. 1).

**Dex had no Effects on the Immunomodulation of iPSC-MSCs on the Local Inflammation in CHS Model**

We previously found that iPSC-MSCs could suppress Th2 response in a mouse model of allergic inflammation. To determine whether Dex influences the immunomodulatory function of iPSC-MSCs in allergic inflammation, a mouse model of CHS, in which Th2-like reaction is involved, was developed (Fig. 3(a)). Firstly, we investigated whether Dex treatment affects the immunomodulatory function of lymph node’s weight. As expected, there was a significant enhancement of lymph node’s weight in CHS models compared with control mice (Fig. 3(b)). Mouse with the treatment of iPSC-MSCs or Dex alone had a marked reduction of lymph node weight (Fig. 3(b)). Interestingly, there were no differences in lymph node weight between mice with the treatment of iPSC-MSCs alone and iPSC-MSCs combined with Dex (Fig. 4(b)). These data suggested that simultaneous treatment with Dex does not affect the immunomodulatory effects of iPSC-MSCs on the inflammation in regional draining lymph nodes in the CHS model.

Next, we qualitatively and quantitatively compared the effects of iPSC-MSCs with or without Dex treatment on the histopathology of the external ear. The results showed that there was a significant infiltration of inflammatory cells in the external ear of CHS mice, and the appearance of the ear was redder and more swollen than the control mice (Fig. 3(c–d)). As expected, both iPSC-MSCs and Dex treatment alone showed significant attenuation of the external ear inflammation, including swelling, inflammatory infiltration and thickness of the external ear compared with CHS mice (Fig. 3(c–d)). Interestingly, Dex treatment alone showed a stronger immunoinhibitory effect on the ear thickness of...
CHS mice compared with those of iPSC-MSCs alone or combined with Dex (Fig. 3(d)). However, there was no significant difference between iPSC-MSCs alone and iPSC-MSCs combined with Dex (Fig. 3(d)), suggesting that Dex had no effects on the immunomodulation of iPSC-MSCs on the inflammation in CHS mice. Next, we measured the IL-6 and IL-10 levels in the serum of CHS mice. Unexpectedly, the results showed that there was no significant difference in IL-6 and IL-10 levels among the five groups (Supplementary Fig. 2). Taken together, these findings suggested that Dex did not affect the immunomodulation of iPSC-MSCs on the local inflammation in the CHS model.
Co-administration of iPSC-MSCs with Dex Decreased Local Expression of IFN-γ and TNF-α in the CHS Model

After 2 days from challenge in CHS mice, the ear tissues were harvested to determine the expressions of IFN-γ and TNF-α by western blot analysis. CHS mice showed increased expression of IFN-γ and TNF-α compared with those of control mice (Fig. 4). Dex alone decreased the expression of IFN-γ and TNF-α in the ears of CHS mice. Treatment of iPSC-MSCs alone showed no significant effect on both IFN-γ and TNF-α expression. The levels of IFN-γ
and TNF-α in the ears of CHS mice co-administered with iPSC-MSCs and Dex were lower than those of iPSC-MSCs administered alone, but similar to Dex alone, suggesting Dex could inhibit the production of IFN-γ and TNF-α in the CHS model independent of iPSC-MSC transplantation.

**Dex did Not Affect the Immunomodulatory Effect of iPSC-MSCs on Allergic Airway Inflammation in Mice**

To further confirm the effects of Dex on the immunomodulatory effect of iPSC-MSCs in vivo, we developed a mouse model of OVA-induced allergic airway inflammation. OVA-sensitized mice developed a significant pulmonary inflammation as evidenced by higher bronchial and vascular scores in lung histology (Fig. 5(a) and (b)), and increased numbers of total inflammatory cells and eosinophils in BALF (Fig. 5(c) and (d)) compared with those in PBS-sensitized mice. In contrast, iPSC-MSC and Dex treatment, alone and combined, exhibited a significant reduction in pulmonary inflammation compared with those in OVA-sensitized mice treated with PBS (Fig. 5(a–d)). Furthermore, the mice treated with iPSC-MSCs combined with Dex showed a similar extent of reduction in pulmonary inflammation as those treated with iPSC-MSCs or Dex alone. This suggested that Dex had no effects on the immunomodulation of iPSC-MSCs on allergic airway inflammation.
Discussion

The iPSC-MSCs have advantages over BM-MSCs, including longer lifespan, various sources and easier in vitro expansion, which makes it an important source of MSCs. We have successfully derived multipotent MSCs from human iPSCs and demonstrated that human iPSC-MSCs are capable of immunomodulation, such as inhibiting lymphocyte proliferation and suppressing Th2 cytokines including IL-4, IL-5, or IL-13 in a mouse model of allergic airway inflammation, suggesting the potential clinical application of iPSC-MSCs for the treatment of allergic diseases. Glucocorticoids are commonly used to treat allergic diseases. Therefore, the interaction between glucocorticoids and iPSC-MSCs needs to be determined before clinical application. In the present study, we have observed that simultaneous treatment with iPSC-MSCs and Dex showed a comparable immunoinhibitory effect on PBMC proliferation induced by anti-CD3/CD28 in vitro, reduced local inflammation in the ears of CHS mice, inhibited anti-CD3-activated T-cell proliferation in vitro and reduced local inflammation in the ears of CHS mice, as indicated by reduction of gross lymph node weight, inflammatory infiltration and the thickness of the external ear, and decreased allergic airway inflammation in mice as evidenced by lower inflammation scores in the lung and fewer inflammatory cells and eosinophils in BALF. In terms of the mechanisms underlying the action of iPSC-MSCs, we recently reported that iPSC-MSCs expressed a high level of IL-10, an important anti-inflammatory cytokine, to inhibit monocyte differentiation into dendritic cells in vitro. Here we did not observe a significant difference in IL-10 levels in blood between mice treated with and without iPSC-MSCs. Additionally, no difference was observed for IDO expression in iPSC-MSCs after co-culture with PBMCs in vitro. These results suggested that there may be other mechanisms involved in the iPSC-MSC action in the present study.

There have been several studies investigating the effects of immunosuppressive drugs on the viability, proliferation, differentiation and function of MSCs. Mycophenolic acid, a cell cycle inhibitor, and rapamycin, an mTOR inhibitor, have been shown to suppress MSC proliferation at therapeutic doses. High-dose tacrolimus, a calcineurin inhibitor, induced toxicity in adult MSCs, whereas MSCs combined with low-dose tacrolimus was shown to be as effective as standard dose tacrolimus in maintaining graft survival after kidney transplantation, supporting the simultaneous application of MSCs and low-dose tacrolimus.

Only a few studies have investigated the effects of steroids on the immunomodulatory function of MSCs. One study reported that Dex did not antagonize the inhibitory effect of human BM-MSCs in a mixed lymphocyte response. However, a recent study showed that Dex significantly eliminated the immunosuppressive effects of BM-MSCs on anti-CD3-activated T-cell proliferation in vitro and in a mouse model of liver fibrosis, in which Dex inhibited the expression of inducible nitric oxide synthase by preventing STAT1 phosphorylation, thus suggesting that concurrent application of MSCs with steroids should be avoided in clinical settings. However, our findings have shown that Dex had no effects on the immunosuppressive effects of iPSC-MSCs on lymphocyte proliferation, allergic diseases of CHS or airway inflammation. It supported the feasibility of concomitant application of iPSC-MSCs with steroids in some clinical settings. Or sometimes, concomitant application of iPSC-MSCs with a low dosage of steroid may have better results compared with high dosage of steroid. Of course, the discrepancy underlying the interaction between steroids and adult MSCs or iPSC-MSCs should be further investigated.

We observed that the combination of iPSC-MSCs and Dex had less effect on decreasing ear thickness compared with Dex treatment alone in CHS mice. However, no
Fig. 5. Dex did not affect the immunomodulatory effect of iPSC-MSCs on allergic airway inflammation in mice. (a) Representative photomicrographs of H&E stained lung sections from each group (magnification, ×400). (b) Statistical analysis for inflammation score in the lungs. (c) Representative diff-quick staining of the inflammatory cells present in the BALF (magnification, ×400). (d) Total cells and eosinophils counts in the BALF. The results represent three independent experiments, 4–6 animals were evaluated for each group. Data are expressed as means ± SEMs. *P < 0.05, **P < 0.01, ***P < 0.001.

BALF: bronchoalveolar lavage fluid; Dex: dexamethasone; H&E: hematoxylin and eosin; iPSC-MSC: induced pluripotent stem cell-derived mesenchymal stem cell; OVA: ovalbumin; PBS: phosphate-buffered saline.
significant difference was found for the IFN-γ and TNF-α levels in the ears of CHS mice, or the inflammation in the lungs in allergic mice between the combination of iPSC-MSCs and Dex and Dex alone. Anyway, we are unable to exclude a possibility that iPSC-MSCs might have a disruptive effect on the anti-inflammatory effects of Dex in some settings. Their interaction should be carefully addressed in specific settings. Fortunately, we found that although Dex was able to inhibit PBMC proliferation in a concentration-dependent manner (from 0.01 to 100 μg/ml), it showed a consistent suppressive effect on PBMC proliferation when co-cultured with iPSC-MSCs, which was equivalent to that of iPSC-MSCs in the absence of Dex. It suggested good application of iPSC-MSCs combined with an even low dosage of steroid.

We acknowledge some limitations in our current study. Although we determined the effect of iPSC-MSCs combined with Dex on local inflammation in CHS mice, we did not evaluate the differential subsets of inflammatory cells, which should be further assessed by flow cytometry analysis. Secondly, we acknowledge the possibility that iPSC-MSCs might interrupt the inhibitory effect of Dex on lymphocyte proliferation and in CHS mice in our study, which should be further studied in glucocorticoid receptor knockout mice in the future.

In conclusion, to our knowledge, we provide the first preliminary evidence that Dex does not significantly affect the immunomodulatory function of iPSC-MSCs both in vitro and in vivo. It supports the feasibility of concomitant application of iPSC-MSCs with steroids in clinical settings.

Acknowledgments
Dan Wang and Yue-Qi Sun contributed equally for this article. Dan Wang and Yue-Qi Sun performed the cell culture, collection and assembly of data, data analysis, and prepared the manuscript; Wen-Xiang Gao and Dan Wang performed flow cytometry, western blot, and preparation of stem cells; Xing-Liang Fan edited the manuscript. Jian-Bo Shi and Qing-Ling Fu contributed to the conception and design of the study. Qing-Ling Fu wrote and revised the manuscript. All authors reviewed and approved the final manuscript.

Ethical Approval
The study protocol was approved by the Ethics Committee of the First Affiliated Hospital, Sun Yat-sen University (No. 2014C-053 for human) and Institutional Animal Care and Use Committee Sun Yat-sen University, China (No. DB-16-0404 for animals).

Statement of Human and Animal Rights
All procedures were performed according to protocols approved by the Institutional Animal Care and Use Committee, Sun Yat-sen University.

Statement of Informed Consent
Human blood buffy coats from ‘anonymous donors’ were obtained from Guangzhou Blood and an exemption of the written informed consent was approved.

Declaration of Conflicting Interests
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The study was supported by grants from the National Natural Science Foundation of China (No. 81500768, 81322012, 81373174, 81273272, 81470069, 81670902, 81770984) and the Science and Technology Foundation of Guangdong Province of China (No. 2015B020225001, 2016A020215049, 2016A030308017).

Supplemental Material
Supplemental material for this article is available online.

References
1. Singer NG, Caplan Al. Mesenchymal stem cells: Mechanisms of inflammation. Annu Rev Pathol. 2011;6:457–478.
2. Lazarus HM, Koc ON, Devine SM, Curtin P, Maziarz RT, Holland HK, Shpall EJ, McCarthy P, Atkinson K, Cooper BW, Gerson SL, Laughlin MJ, Loberiza FR Jr., Moseley AB, Bacigalupo A. Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients. Biology of blood and marrow transplantation: J Am Soc Blood Marrow Transplantat. 2005;11(5):389–398.
3. Sun LY, Wang DD, Liang J, Zhang HY, Feng XB, Wang H, Hua BZ, Liu BJ, Ye SQ, Hu XA, Xu WR, Zeng XF, Hou YY, Gilkeson GS, Silver RM, Lu LW, Shi ST. Umbilical cord mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus. Arthritis Rheum. 2010;62(8):2467–2475.
4. Garcia-Olmo D, Garcia-Arranz M, Herreros D, Pascual I, Peiro C, Rodriguez-Montes JA. A phase I clinical trial of the treatment of Crohn’s fistula by adipose mesenchymal stem cell transplantation. Dis Colon Rectum. 2005;48(7):1416–1423.
5. Squillaro T, Peluso G, Galderisi U. Clinical trials with mesenchymal stem cells: An update. Cell Transplant. 2016;25(5):829–848.
6. Faiella W, Atoui R. Immunotolerant properties of mesenchymal stem cells: Updated review. Stem Cells Int. 2016;2016:1859567.
7. Uccelli A, Pistoia V, Moretta L. Mesenchymal stem cells: A new strategy for immunosuppression? Trends Immunol. 2007;28(5):219–226.
8. Bonfield TL, Nolan Koloze MT, Lennon DP, Caplan AI. Defining human mesenchymal stem cell efficacy in vivo. J Inflamm (Lond). 2010;7:51.
9. Zhang Y, Liang X, Lian Q, Tse HF. Perspective and challenges of mesenchymal stem cells for cardiovascular regeneration. Expert Rev Cardiovasc Ther. 2013;11(4):505–517.
10. Xin Y, Wang YM, Zhang H, Li J, Wang W, Wei YJ, Hu SS. Aging adversely impacts biological properties of human bone marrow-derived mesenchymal stem cells: Implications for
tissue engineering heart valve construction. Artif Organs. 2010;34(3):215–222.
11. Zaim M, Karaman S, Cetin G, Isik S. Donor age and long-term culture affect differentiation and proliferation of human bone marrow mesenchymal stem cells. Ann Hematol. 2012;91(8):1175–1186.
12. Sepulveda JC, Tome M, Fernandez ME, Delgado M, Campisi J, Bernad A, Gonzalez MA. Cell senescence abrogates the therapeutic potential of human mesenchymal stem cells in the lethal endotoxemia model. Stem Cell. 2014;32(7):1865–1877.
13. Takahashi K, Yamanaka S. A decade of transcription factor-mediated reprogramming to pluripotency. Nat Rev Mol Cell Biol. 2016;17(3):183–193.
14. Amabile G, Meissner A. Induced pluripotent stem cells: current progress and potential for regenerative medicine. Trends Mol Med. 2009;15(2):59–68.
15. Nishikawa S, Goldstein RA, Nierras CR. The promise of human induced pluripotent stem cells for research and therapy. Nat Rev Mol Cell Biol. 2008;9(9):725–729.
16. Yamana S. Strategies and new developments in the generation of patient-specific pluripotent stem cells. Cell Stem Cell. 2007;1(1):39–49.
17. Lin L, Bolund L, Luo Y. 2016. Towards personalized regenerative cell therapy: Mesenchymal stem cells derived from human induced pluripotent stem cells. Curr Stem Cell Res Ther. 11(2):122–130.
18. Jung Y, Bauer G, Nolta JA. Concise review: Induced pluripotent stem cell-derived mesenchymal stem cells: Progress toward safe clinical products. Stem Cell. 2012;30(1):42–47.
19. Lian Q, Zhang Y, Zhang J, Zhang HK, Wu X, Zhang Y, Lam FF, Kang S, Xia JC, Lai WH, Au KW, Chow YY, Siu CW, Lee CN, Tse HF. Functional mesenchymal stem cells derived from human induced pluripotent stem cells attenuate limb ischemia in mice. Circulation. 2010;121(9):1113–1123.
20. Fu QL, Chow YY, Sun SJ, Zeng QX, Li HB, Shi JB, Sun YQ, Wen W, Tse HF, Lian Q, Xu G. Mesenchymal stem cells derived from human induced pluripotent stem cells modulate T-cell phenotypes in allergic rhinitis. Allergy. 2012;67(10):1215–1222.
21. Sun YQ, Deng MX, He J, Zeng QX, Wen W, Wong DS, Tse HF, Xu G, Lian Q, Shi J, Fu QL. Human pluripotent stem cell-derived mesenchymal stem cells prevent allergic airway inflammation in mice. Stem Cell. 2012;30(12):2692–2699.
22. Giuliani M, Oudhri N, Noman ZM, Vernochet A, Chouaib S, Azzarone B, Durbach A, Benaicour-Griscelli A. Human mesenchymal stem cells derived from induced pluripotent stem cells down-regulate NK-cell cytolytic machinery. Blood. 2011; 118(12):3254–3262.
23. Chen YS, Pelekanos RA, Ellis RL, Horne R, Wolutang EJ, Fisk NM. Small molecule mesenengenic induction of human induced pluripotent stem cells to generate mesenchymal stem/stromal cells. Stem Cell Transl Med. 2012;1(2):83–95.
24. Hynes K, Menicanin D, Mrozik K, Gronthos S, Bartold PM. Generation of functional mesenchymal stem cells from different induced pluripotent stem cell lines. Stem Cell Develop. 2014;23(10):1084–1096.
25. Kimbrel EA, Kouris NA, Yavarian GM, Chu J, Qin Y, Chan A, Singh RP, McCurdy D, Gordon L, Levinson RD, Lanza R. Mesenchymal stem cell population derived from human pluripotent stem cells displays potent immunomodulatory and therapeutic properties. Stem Cell Develop. 2014;23(14):1611–1624.
26. Zhang Y, Liang X, Liao S, Wang W, Wang J, Li X, Ding Y, Liang Y, Gao F, Yang M, Fu Q, Xu A, Chai YH, He J, Tse HF, Lian Q. Potent paracrine effects of human induced pluripotent stem cell-derived mesenchymal stem cells attenuate doxorubicin-induced cardiomyopathy. Sci Rep. 2015;5: 11235.
27. Knyazev OV, Parfenov AI, Konopolyanikov AG, Boldyreva ON. [Use of mesenchymal stem cells in the combination therapy of ulcerative colitis]. Ter Arkh. 2016;88(2):44–48.
28. Peng Y, Ke M, Xu L, Liu L, Chen X, Xia W, Li X, Chen Z, Ma J, Liao D, Li G, Fang J, Pan G, Xiang AP. Donor-derived mesenchymal stem cells combined with low-dose tacrolimus prevent acute rejection after renal transplantation: A clinical pilot study. Transplantation. 2013;95(1):161–168.
29. Perico N, Casiraghi F, Introna M, Gotti E, Todeschini M, Cavainato RA, Capelli C, Rambaldi A, Cassis P, Rizzo P, Cortinovis M, Marasa M, Golay J, Noris M, Remuzzi G. Autologous mesenchymal stromal cells and kidney transplantation: A pilot study of safety and clinical feasibility. Clin J Am Soc Nephrol. 2011;6(2):412–422.
30. Yin F, Battiwalla M, Ito S, Feng X, Chinian F, Melenhorst JJ, Koklanaris E, Sabatino M, Stoneck D, Samsel L, Klotz J, Hensel NF, Robey PG, Barrett AJ. Bone marrow mesenchymal stromal cells to treat tissue damage in allogeneic stem cell transplant recipients: Correlation of biological markers with clinical responses. Stem Cell. 2014;32(5):1278–1288.
31. Li JF, Zhang DJ, Geng T, Chen L, Huang H, Yin HL, Zhang YZ, Lou JY, Cao B, Wang YL. The potential of human umbilical cord-derived mesenchymal stem cells as a novel cellular therapy for multiple sclerosis. Cell Transplant. 2014;23(Suppl. 1):S113–S122.
32. Buron F, Perrin H, Malcus C, Hequet O, Thaunat O, Kholopova MN, Moulin FT, Morelon E. Human mesenchymal stem cells and immunosuppressive drug interactions in allogeneic responses: An in vitro study using human cells. Transplant Proc. 2009;41(8):3347–3352.
33. Chen X, Gan Y, Li W, Su J, Zhang Y, Huang Y, Roberts Al, Han Y, Li J, Wang Y, Shi Y. The interaction between mesenchymal stem cells and steroids during inflammation. Cell Death Dis. 2014;5:e1009.
34. Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, Lanino E, Sundberg B, Marzano M, Remberger M, Dini G, Egeler RM, Bacigalupo A, Fiebke W, Ringden O, Developmental Committee of the European Group for Blood, Marrow T. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: A phase II study. Lancet. 2008;371(9624):1579–1586.
35. Su WR, Zhang QZ, Shi SH, Nguyen AL, Le AD. Human gingiva-derived mesenchymal stromal cells attenuate contact
hypersensitivity via prostaglandin e2-dependent mechanisms.

36. Keating A. Mesenchymal stromal cells: New directions. Cell Stem Cell. 2012;10(6):709–716.

37. Hayashi T, Hara S, Hasegawa K. Enhanced contact hypersensitivity by delayed T-helper 2 response in BALB/C mice. Allergy Asthma Proc. 2009;30(4):449–457.

38. Whitt J, Vallabhaneni KC, Penfornis P, Pochampally R. Induced pluripotent stem cell-derived mesenchymal stem cells: a leap toward personalized therapies. Curr Stem Cell Res Ther. 2016;11(2):141–148.

39. LeBlanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, Lanino E, Sundberg B, Bernardo ME, Remberger M, Dini G, Egeler RM, Bacigalupo A, Fibbe W, Ringden O. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: A phase II study. Lancet. 2008;371(9624):1579–1586.

40. Wu KH, Chan CK, Tsai C, Chang YH, Sieber M, Chiu TH, Ho M, Peng CT, Wu HP, Huang JL. Effective treatment of severe steroid-resistant acute graft-versus-host disease with umbilical cord-derived mesenchymal stem cells. Transplantation. 2011;91(12):1412–1416.

41. Gao WX, Sun YQ, Shi J, Li CL, Fang SB, Wang D, Deng XQ, Wen W, Fu QL. Effects of mesenchymal stem cells from human induced pluripotent stem cells on differentiation, maturation, and function of dendritic cells. Stem Cell Res Ther. 2017;8(1):48.

42. Hoogduijn MJ, Crop MJ, Korevaar SS, Peeters AM, Eijken M, Maat LP, Balk AH, Weimar W, Baan CC. Susceptibility of human mesenchymal stem cells to tacrolimus, mycophenolic acid, and rapamycin. Transplantation. 2008;86(9):1283–1291.

43. Pan GH, Chen Z, Xu L, Zhu JH, Xiang P, Ma JJ, Peng YW, Li GH, Chen XY, Fang JL, Guo YH, Zhang L, Liu LS. Low-dose tacrolimus combined with donor-derived mesenchymal stem cells after renal transplantation: A prospective, non-randomized study. Oncotarget. 2016;7(11):12089–12101.