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Human adipose, placenta, and umbilical cord-derived mesenchymal stem cells ameliorate imiquimod-induced psoriatic mice via reducing T cells infiltration

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Abstract: Psoriasis is an autoimmune-related chronic inflammatory disease with an approximate prevalence of 2–3% around the world, involving increased keratinocyte proliferation. Indeed, Th17 cells and IL-17 play critical roles in the pathogenesis of psoriasis. The monoclonal antibodies against cytokines have been shown to have effectively immunosuppressive effects on human psoriasis. However, there are still some patients that have no response to these treatments. Some patients have even serious side-effects which may affect their life. Mesenchymal stem cells have the ability of immunosuppressive and anti-inflammatory effects, which may be an alternative therapy with more safety and efficacy for human psoriasis. Moreover, the underlying mechanisms by which the MSCs prevent or ameliorate psoriasis are still poorly understood. Here, we first isolated and characterized human adipose, placenta, and umbilical cord-derived mesenchymal stem cells (haMSCs, hpMSCs, and huMSCs). After that, the animal model of imiquimod (IMQ)-induced psoriasis in C57BL/6 mice was confirmed. We investigated the impact of haMSCs, hpMSCs, and huMSCs on this model by H&E staining, immunohistochemistry staining, and quantitative real-time PCR. Data analysis showed that mice subcutaneously injected with these MSCs had a significantly decreased epidermal thickness, which was caused by obviously reduced hyper-proliferation of keratinocytes. Furthermore, our findings revealed that the infiltration of T cells to psoriatic lesions in IMQ-induced psoriasis mice was markedly downregulated by intradermal administration of haMSCs, hpMSCs, and huMSCs, respectively. Consequently, the production of IL-17 from Th17 cells was reduced, which inhibits the proliferation of keratinocytes in lesioned skin of IMQ-induced psoriasis mice. These data suggest that haMSCs, hpMSCs, and huMSCs can inhibit the effects of proinflammatory Th17 cells on the development of psoriasis, which may be potential therapeutic candidates for skin inflammatory disease or other autoimmune diseases.

Introduction

Psoriasis is an autoimmune-related chronic inflammatory disease, which can represent various phenotypes on the skin, and has a complex genetic architecture (Lebwohl, 2003). Psoriatic patients are widely distributed around the world, with an approximate prevalence of 2–3% (Greb et al., 2016). Generally, psoriasis manifest as various phenotypes that include vulgaris psoriasis, guttate psoriasis, inverse psoriasis, pustular psoriasis, palmoplantar psoriasis, and erythrodermic psoriasis, in which vulgaris psoriasis is the main manifestation (Greb et al., 2016). The pathological characteristics of psoriatic skin mainly manifest as epidermal hyperplasia, increased angiogenesis, dilated capillary vessels, increased immune cell infiltration, and related cytokines and chemokines (Lowes et al., 2007; Griffiths and Barker, 2007). Psoriasis is strongly associated with proinflammatory T cells, in which T-helper 1 (Th1) and T-helper 17 (Th17) cells play an important role in the pathogenesis of psoriasis (Lowes et al., 2008). However, Th17 cells are proposed to be a major player in that IL-17 secreted from Th17 cells is a crucial proinflammatory cytokine in psoriasis (Di Cesare et al., 2009). The skin inflammation in psoriasis is caused by T cells and neutrophils infiltration into

the dermis and epidermis, which reduces the antioxidant capacity of the skin (Sah *et al.*, 2016).

Imiquimod (IMQ) is a ligand for toll-like receptor 7 and 8 (TLR7/8) and has been reported to induce psoriasis-like inflammatory skin disease via activating the IL23/IL17 axis, which partially dependent on the presence of T cells (van der Fits *et al.*, 2009). Moreover, the topical application of IMQ increases the level of relative chemokines and pro-inflammatory cytokines in the derma. These features are in accordance with that of psoriasis in patients. Therefore, IMQ-induced psoriasis in mice can closely resemble human psoriasis (van der Fits *et al.*, 2009), and this mouse model is generally used to evaluate the therapeutic effects of some drugs on psoriasis (Li *et al.*, 2020). Additionally, there are also many psoriatic mouse models, which included xenograft models, spontaneous models, transgenic models, and IL-23-induced models (Bocheńska *et al.*, 2017).

Currently, therapeutic approaches of biologics and cell transplantation are being developed and explored. In previous reports, the drugs of monoclonal antibodies against cytokines have been shown to have effectively immunosuppressive effects on human psoriasis (Di Cesare *et al.*, 2009). However, some patients have no response to the treatment of monoclonal antibody drugs, and their therapeutic effect is low or ineffective (Gedebjerg *et al.*, 2013). Moreover, during these antibody drugs treatment, some patients have common side-effects, even serious side-effects which may affect their life, including hypertension, heart failure, and liver dysfunction (Jeon *et al.*, 2017a). Thus, it is urgent to develop an alternative therapy with more safety and efficacy for human psoriasis.

Mesenchymal stem cells (MSCs) are multipotent progenitor cells, which have strong immunosuppressive and anti-inflammatory effects through the interaction with immune cells or soluble factors secreted from MSCs (Su *et al.*, 2011). Various studies have indicated that the MSCs derived from bone marrow, human umbilical cord blood, human palatine tonsil, and human embryonic stem cell could be used for prevention or treatment of autoimmune diseases in mice, such as encephalomyelitis (Zappia *et al.*, 2005), arthritis (Augello *et al.*, 2007), atopic dermatitis (Na *et al.*, 2014), psoriasis (Sah *et al.*, 2016; Lee *et al.*, 2017; Cho *et al.*, 2017; Kim *et al.*, 2019). However, above these MSCs are not easily available, which does not benefit for the large-scale production and MSCs-based therapeutics for clinical use. In addition, the underlying mechanisms by which the MSCs prevent or ameliorate psoriasis are still poorly understood. In this study, we aimed to explore the effects of human adipose, placenta, and umbilical cord-derived mesenchymal stem cells (haMSCs, hpMSCs, and huMSCs) on IMQ-induced psoriasis in mice and their possible mechanisms.

Materials and Methods

Isolation and culture of haMSCs, hpMSCs, and huMSCs
The haMSCs were isolated from lipoaspirates and cultured as the previous description (Wang *et al.*, 2015). The human placenta and umbilical cord were obtained with informed consent from healthy mothers undergoing cesarean section. The procedure was approved by the Institutional Patients and Ethics Committee of Shanghai Baijia Maternity Hospital (Shanghai, China). Human placenta and umbilical cord-derived mesenchymal stem cells (hpMSCs and huMSCs) were isolated according to the modified method (Ikhsan *et al.*, 2020; Putra *et al.*, 2020). In brief, the tissue of the human placenta and umbilical cord were rinsed to remove the blood cells with phosphate-buffered saline (PBS) containing 1% penicillin/streptomycin solution and then cut into ~25 mm^2 pieces with scissors. Subsequently, these pieces were resuspended in 20 mL PBS containing 0.2% Type II collagenase (Gibco, #17101-015, USA) digestion and incubated on a shaker at 37°C for 2 h. The supernatant was filtered through a 100 μm cell sieve (BD Biosciences, San Jose, CA, USA). The filtrate was centrifuged at $300 \times g$ for 10 min. Finally, the cell pellets were resuspended and cultured in a T75 flask with α-MEM medium (Gibco, C12571500BT, USA) supplemented with 5% EliteGro (EliteCell, EPAGMP-500, USA) in a humidified incubator at 37°C with 5% CO_2. After culturing for 24 h, the unattached cells were removed. The fresh medium was added to the remaining cells, which were defined as passage 0 (P0). The medium was changed every 2 days. When they were cultured to the confluence of 80–90%, the cells were trypsinized and sub-cultured. The hpMSCs and huMSCs at P3-P4 were harvested for animal study.

Flow cytometry analysis
The MSCs of 1×10^6 were resuspended in 100 μL PBS and incubated with 1 μg phycoerythrin (PE)-conjugated, allophycocyanin (APC)-conjugated, fluorescein isothiocyanate (FITC)-conjugated, or peridinin-chlorophyll-protein (PerCP)-Cy5.5- conjugated anti-human monoclonal antibodies in the dark for 30 min at 4°C. These antibodies included the following: CD34 (Biolegend, #343503, USA), CD45 (Biolegend, #304008, USA), CD73 (Biolegend, #344016, USA), CD90 (Biolegend, #328118, USA), CD105 (Biolegend, #800508, USA), and HLA-DR (Biolegend, #307603, USA). Subsequently, These MSCs were washed with cold PBS and then analyzed by flow cytometry (BD Biosciences, San Jose, CA, USA).

Trilineage differentiation of haMSCs, hpMSCs, and huMSCs in vitro
Adipogenesis, osteogenesis, and chondrogenesis of haMSCs, hpMSCs, and huMSCs were respectively determined as the previous description (Ma *et al.*, 2019). Briefly, the MSCs at passage 4 were seeded onto 6-well plates at 1×10^4 cells per cm^2 for adipogenesis and osteogenesis and maintained with the culture medium in a humidified incubator at 37°C with 5% CO_2. When the MSCs had a 90% confluency, the culture medium was replaced by adipogenic or osteogenic differentiation medium (Gibco, USA) and kept for 3 weeks. For chondrogenic differentiation, 1×10^6 cells resuspended in 1 mL chondrogenic differentiation medium were centrifuged at $300 \times g$ for 5 min and kept in conical tubes for 4 weeks. Samples were fixed in 4% paraformaldehyde solution for 30 min. Positive induction of adipogenesis and osteogenesis was respectively confirmed by Oil Red O staining (Sciencell, SC-0843, USA) and Alizarin Red staining (Sciencell, SC-0223, USA). Cryosection of a chondrogenic pellet was carried out, and the section was stained with Safranin O staining (Sciencell, SC-8348, USA).

Mice and treatments

C57BL/6 wild-type male mice were purchased from Shanghai SLAC laboratory animal center (Shanghai, China). All mice used for the study at 8 weeks of age were provided with food and water under specific pathogen-free conditions on a 12-hours light/dark cycle. All experiments were performed in accordance with the animal ethics committee of the Scientific Investigation Board of Shanghai Jiaotong University School of Medicine, Shanghai, China (IACUC approval No. 2019-A021-01). Age- and sex-matched mice were randomly divided into five groups of six mice each. Mice were received a daily topical dose of 62.5 mg of commercially available IMQ cream (5% the active compound) (MedShine, #120503, China) on shaved back skin for 7 consecutive days (van der Fits *et al.*, 2009), excluded the blank control mice. The 100 μL of 2×10^6 MSCs suspended in the saline (Lee *et al.*, 2017) or saline was subcutaneously injected into the back of IMQ-treated mice using a 26-gauge needle on day −1, 3, and 5, respectively. Meanwhile, negative control mice received a subcutaneous injection of an equal volume of saline. Finally, after mice were sacrificed on day 7, the samples of dorsal skin were collected for analysis.

Histological analysis and immunohistochemistry

Skin samples were fixed in 4% paraformaldehyde for 24 h. After washed with distilled water, the samples were dehydrated in a graded ethanol series and then embedded in paraffin (Jeon *et al.*, 2017b). 5 μm-thick sections on slides were used for hematoxylin and eosin (H&E) and immunohistochemistry staining. Epidermal hyperplasia (acanthosis) was assessed using the average area of measurements of five random fields per section. Briefly, the epidermal area was outlined and measured by the lasso tool in Adobe Photoshop CS4 (Yan *et al.*, 2015). And the epidermal thickness was measured as the average of 20 random measures of five random fields per section between stratum basale and stratum granulosum, excluding the stratum corneum and hair follicles.

For immunohistochemistry staining, sections were incubated with primary rabbit monoclonal anti-mouse CD3 (1:150, Abcam, ab16669, USA) or rabbit polyclonal anti-mouse Ki67 (1:200, Abcam, ab15580, USA) antibody at 4°C overnight, and then with diluted biotin-conjugated anti-rabbit IgG at room temperature following the manufacturer's instructions. The twenty-four images of stained sections of each sample were randomly captured by light microscopy (Axioscope A1, Zeiss). The number of dermal infiltrating cells, CD3$^+$ cells, and epidermal Ki67$^+$ cells per field was calculated using Adobe Photoshop CS4.

Real-time quantitative PCR

Total RNA of skin samples was extracted using the RNAiso Plus (TaKaRa, #9108/9109, Japan) and quantified by NanoDrop spectrophotometer (ND-1000). And then, 1 μg of total RNA was reverse transcribed into complementary DNA (cDNA) using HiScript$^®$ III RT SuperMix (Vazyme, R323-01, China). 50 ng of cDNA per well was distributed on plates and run in triplicate. Real-time PCR was carried out with TaqMan$^®$ Gene Expression Master Mix (ThermoFisher, #4369016, USA) and TaqMan probes (ThermoFisher, USA) of IL-17 (Mm00439618_m1) and GAPDH (Mm99999915_g1) in a QuantStudio^{TM} DX real-time PCR instrument (Applied Biosystems). The PCR program consisted of 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The relative expression of target genes was calculated and normalized to GAPDH expression by the $2^{-\Delta\Delta CT}$ method (Yan *et al.*, 2015).

Statistical analysis

The data were analyzed with GraphPad Prism 5.0 (GraphPad Software Inc.) and expressed as the mean ± SEM. The unpaired two-tailed Student's *t*-test was used for statistical comparisons between two groups. One-way analysis of variance (ANOVA) followed by *post hoc* Tukey test was used for multiple groups. *p* values of <0.05 were considered statistically significant.

Results

Isolation and characterization of human adipose, placenta, and umbilical cord-derived mesenchymal stem cells

To investigate the effects of haMSCs, huMSCs, and hpMSCs on IMQ-induced psoriasis in mice, we first isolated and characterized these mesenchymal stem cells. Flow cytometry analysis showed that the population of haMSCs, huMSCs, and hpMSCs positively expressed (≥95%) CD73, CD90, and CD105, and negatively expressed (≤2%) CD34, CD45, and HLA-DR (Fig. 1A). Furthermore, the results of trilineage differentiation of haMSCs, huMSCs, and hpMSCs displayed that they could be differentiated toward adipocytes, osteocytes, and chondrocytes in specified differentiation media, respectively (Fig. 1B). Therefore, these findings demonstrate that the isolated cells were confirmed to the characterization of haMSCs, huMSCs, and hpMSCs, respectively.

Establishment of IMQ-induced psoriasis in mice

To determine whether haMSCs, huMSCs, and hpMSCs can prevent psoriasis-like skin inflammation, we first confirmed the animal model of IMQ-induced psoriasis in C57BL/6 mice. (Fig. 2A). Our results showed the back skin became scaly and thickened in IMQ-induced psoriasis mice (Fig. 2B). Quantitative analysis of H&E-stained sections showed that the epidermal thickness was significantly increased in IMQ-induced psoriasis mice compared with control mice (*p* < 0.001, Figs. 2C and 2D). Moreover, in contrast with control mice, the number of dermal infiltrating cells was also markedly enhanced in IMQ-induced psoriasis mice (*p* < 0.001, Fig. 2E). Thus, these results indicate that the animal model of IMQ-induced psoriasis in C57BL/6 mice was successfully confirmed.

Human adipose, placenta, and umbilical cord-derived mesenchymal stem cells inhibit epidermal hyperplasia in the skin of IMQ-induced psoriasis mice

In order to examine the inhibitory effects of haMSCs, huMSCs, and hpMSCs on psoriasis development, these MSCs were subcutaneously injected on days −1, 3, and 5, respectively, and psoriasis was induced by imiquimod for 7 consecutive days (Fig. 3A). The observation of the back skin in mice showed that these MSCs may improve the appearance of psoriasis to a certain extent (Fig. 3B). Subsequently, histopathological analysis of dorsal skin samples was performed by H&E staining and immunohistochemistry staining. Compared with IMQ and saline-treated mice, a
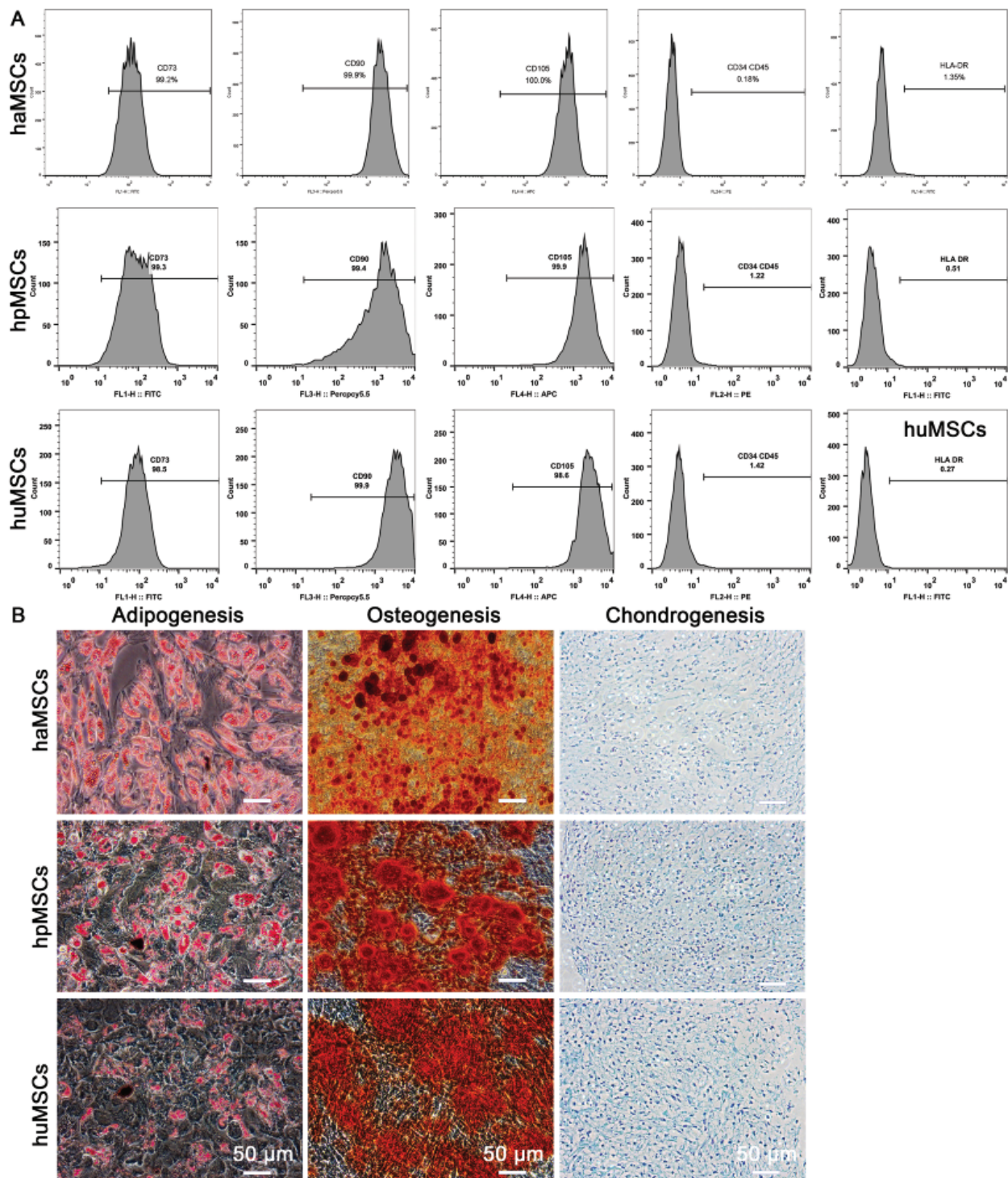
FIGURE 1. Isolation and characterization of human adipose, placenta, and umbilical cord-derived mesenchymal stem cells (haMSCs, hpMSCs, and huMSCs).
(A) Flow cytometry analysis of haMSCs, huMSCs, and hpMSCs expressing marker proteins. The cells were positive for CD73, CD90, CD105, and negative for CD34, CD45, and HLA-DR. (B) Trilineage differentiation of haMSCs, huMSCs, and hpMSCs toward adipocytes, osteocytes, and chondrocytes in respectively specified differentiation media. Scale bar: 50 μm.

reduced epidermal thickness of IMQ-induced mice subcutaneously injected with haMSCs, huMSCs or hpMSCs was observed (Fig. 3C). Moreover, quantitative analysis of epidermal thickness showed that mice treated with haMSCs ($p < 0.05$), huMSCs ($p < 0.01$) or hpMSCs ($p < 0.01$) had a significantly decreased epidermal thickness, compared with saline-treated mice (Fig. 3D). Interestingly, ameliorative effects of huMSCs and hpMSCs on IMQ-induced epidermal thickness seemed to

be more effective than that of haMSCs-treated mice, but there were no significant differences among them ($p > 0.05$, Fig. 3D).

Human adipose, placenta, and umbilical cord-derived mesenchymal stem cells inhibit keratinocyte proliferation in the skin of IMQ-induced psoriasis mice
Psoriasis is characterized by keratinocyte proliferation (Perera *et al.,* 2012). Thus, we explored the level of keratinocyte
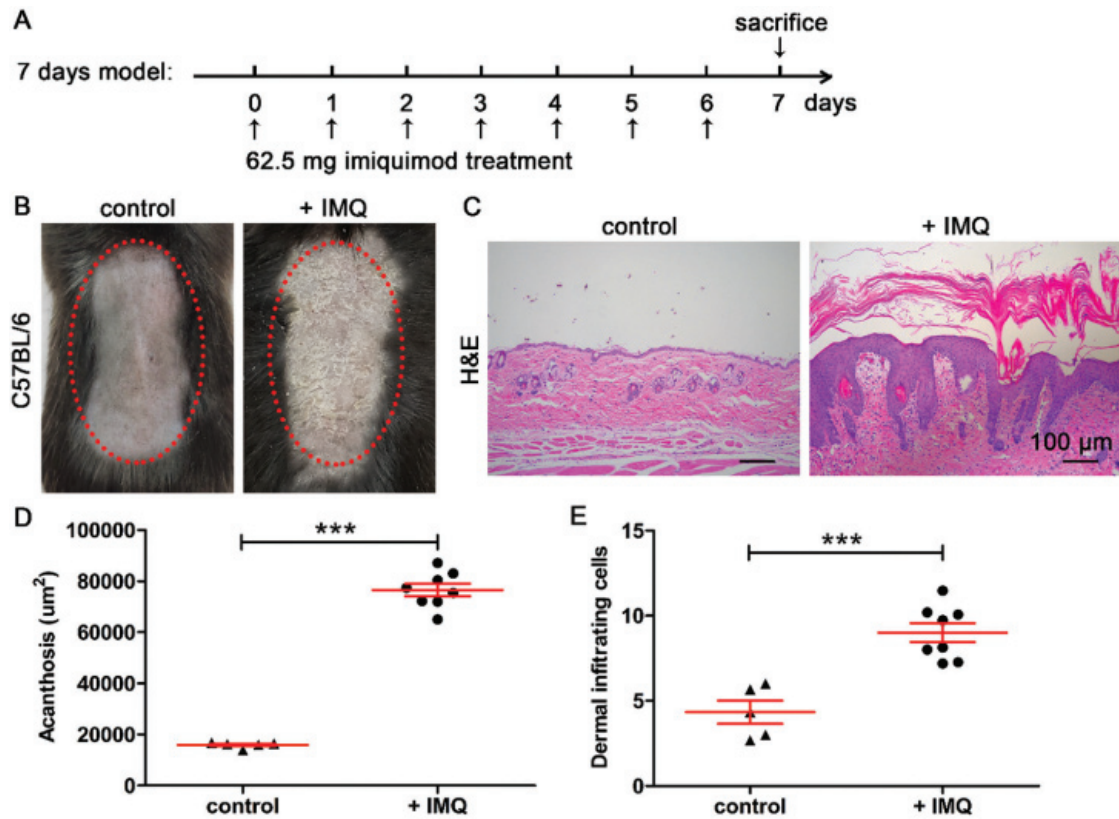
FIGURE 2. Establishment of IMQ-induced psoriasis model in mice.
(A) Schematic diagram of IMQ-induced psoriasis in mice. (B) Phenotypical presentation of shaved back skin of mice with and without IMQ application for 7 consecutive days. (C) H&E staining of shaved back skin of mice with and without IMQ treatment. Scale bar: 100 μm. (D, E) Quantitative analysis of epidermal hyperplasia and dermal infiltrating cells. ***$p < 0.001$, N = 5–8 mice per group.

proliferation in the epidermis of IMQ-induced psoriasis mice by immunohistochemistry staining of Ki67. The data revealed that mice subcutaneously injected with haMSCs, huMSCs or hpMSCs displayed a reduced hyper-proliferation of keratinocytes in the basal layer of the epidermis, compared with saline-treated mice (Fig. 4A). And quantitative analysis of the number of Ki67+ cells per field in the epidermis of haMSCs, huMSCs or hpMSCs-treated mice also revealed hyper-proliferation of keratinocytes was obviously reduced, compared with saline-treated mice ($p < 0.01$, Fig. 4B). These results are consistent with that of H&E staining analysis of epidermal hyperplasia. Consequently, these findings indicate that the administration of haMSCs, huMSCs or hpMSCs could effectively inhibit the epidermal hyperplasia of IMQ-induced psoriasis mice.

Human adipose, placenta, and umbilical cord-derived mesenchymal stem cells reduce inflammatory infiltration of T lymphocyte in the derma of IMQ-induced psoriasis mice
To further confirm the ameliorative effects of human adipose, placenta, and umbilical cord-derived mesenchymal stem cells on IMQ-induced psoriasis mice, histopathological analysis of CD3+ T cells infiltration in the derma of dorsal skin samples was conducted by immunohistochemistry staining. We found that mice treated with haMSCs, huMSCs or hpMSCs showed a much lower number of CD3+ cells infiltration in the derma than that of saline-treated mice (Fig. 5A). Furthermore, quantitative analysis of the number of CD3+ T cells infiltration per field in the derma of haMSCs ($p <$

0.01), huMSCs ($p < 0.001$) or hpMSCs ($p < 0.001$)-injected mice also indicated CD3+ cells infiltration was markedly reduced, compared with saline-treated mice (Fig. 5B). Of note, we observed that the inhibited effects of huMSCs and hpMSCs on CD3+ T cells infiltration in the derma of IMQ-induced mice tended to be more obvious in contrast to haMSCs-treated mice (Fig. 5B). However, the statistical differences among them were no significant ($p > 0.05$, Fig. 5B).

Human adipose, placenta, and umbilical cord-derived mesenchymal stem cells reduce IL-17 expression of T lymphocyte in the derma of IMQ-induced psoriasis mice
To examine the levels of IL-17 expression in lesional skin of IMQ-induced psoriasis mice, we carried out a quantitative real-time PCR assay. The data showed that the levels of IL-17 expression were markedly decreased in lesional skin of IMQ-induced psoriasis mice, which were treated with haMSCs ($p < 0.05$), huMSCs ($p < 0.01$) or hpMSCs ($p < 0.01$), compared with saline-treated psoriatic mice (Fig. 5C). Similarly, we also found that the reduced levels of IL-17 expression tended to be more marked in lesioned skin of IMQ-induced psoriasis mice with huMSCs or hpMSCs treatment, compared to haMSCs-treated mice, but the statistical differences among them were no significant ($p > 0.05$, Fig. 5C).

Discussion

Psoriasis is an autoimmune-mediated chronic inflammatory disease. Its important feature is the epidermal hyperplasia
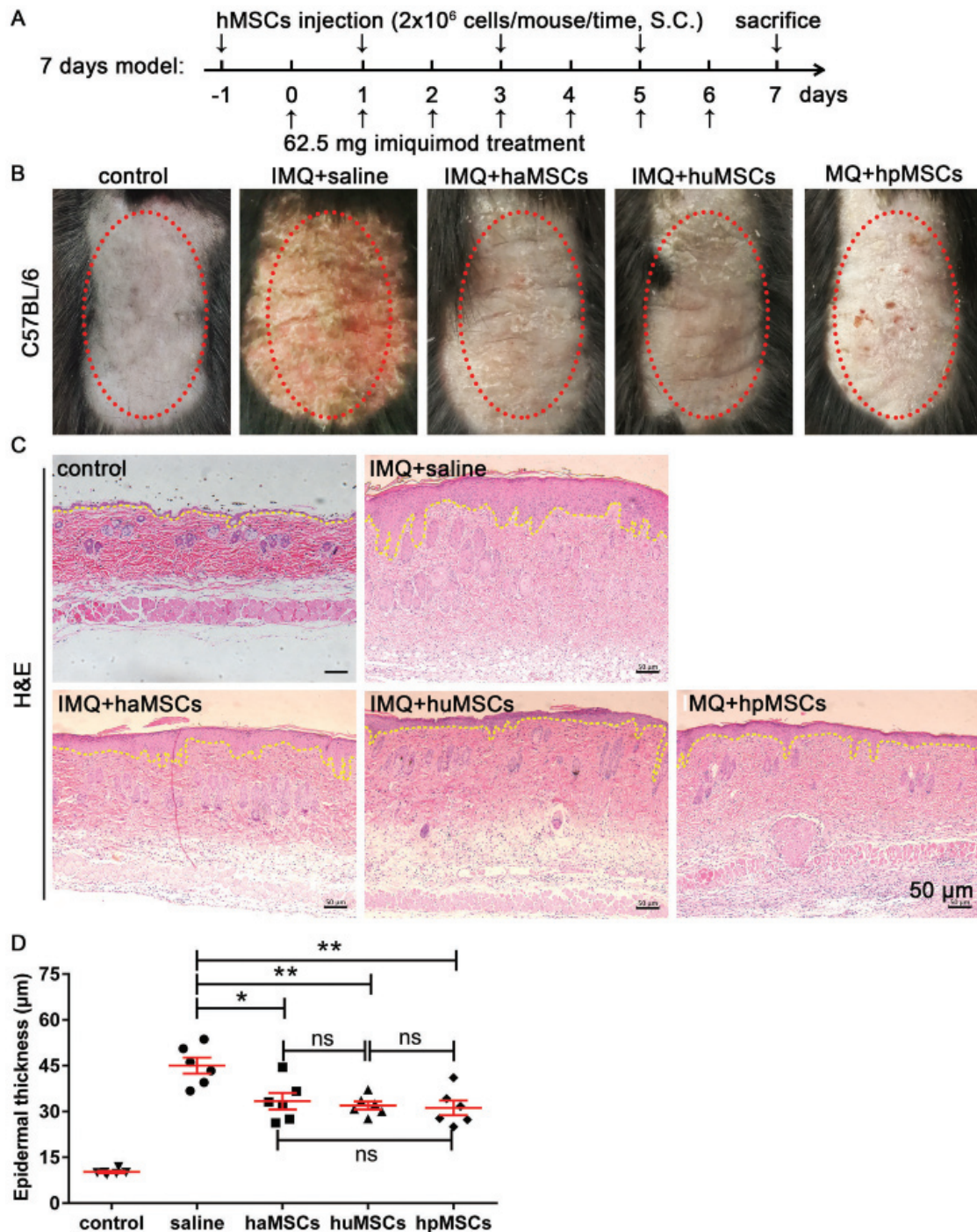
FIGURE 3. Human adipose, placenta, and umbilical cord-derived mesenchymal stem cells reduce epidermal thickness in lesional skin of IMQ-induced psoriasis mice.

(A) Schematic diagram of IMQ-induced psoriasis and treatment with saline, haMSCs, huMSCs or hpMSCs in mice. (B) The observation of the back skin in control and IMQ-induced psoriasis mice treated by saline, haMSCs, huMSCs or hpMSCs. (C) H&E staining of shaved back skin of control and IMQ-treated mice. IMQ-treated mice were subcutaneously injected with saline, haMSCs, huMSCs or hpMSCs, individually. Scale bar: 50 μm. (D) Quantitative analysis of the epidermal thickness in the skin of control and IMQ-treated mice. *$p < 0.05$, **$p < 0.01$, ns, non-significant, N = 6 mice per group.

resulted from keratinocyte excessive proliferation, which is mainly caused by the cytokines released from activated T cells infiltrated into the dermis and epidermis (Lebwohl, 2003). T cells have a critical role in the pathogenesis of psoriasis (Gudjonsson et al., 2004). Psoriasis is strongly associated with the balance of the Th1 and Th17 cells, which play an important role in the pathogenesis of psoriasis (Lowes et al., 2008). However, Th17 cells are proposed to play a pivotal role in psoriatic inflammation in that

IL-17 secreted from Th17 cells is a crucial proinflammatory cytokine in psoriasis (Di Cesare et al., 2009).

A series of studies have revealed that the MSCs derived from human umbilical cord blood (Sah et al., 2016; Lee et al., 2017), palatine tonsil (Cho et al., 2017), and embryonic stem cell (Kim et al., 2019) be applicated to prevent or treat psoriasis in mice by means of their immunosuppressive and anti-inflammatory effects. Additionally, the MSCs isolated from the peripheral
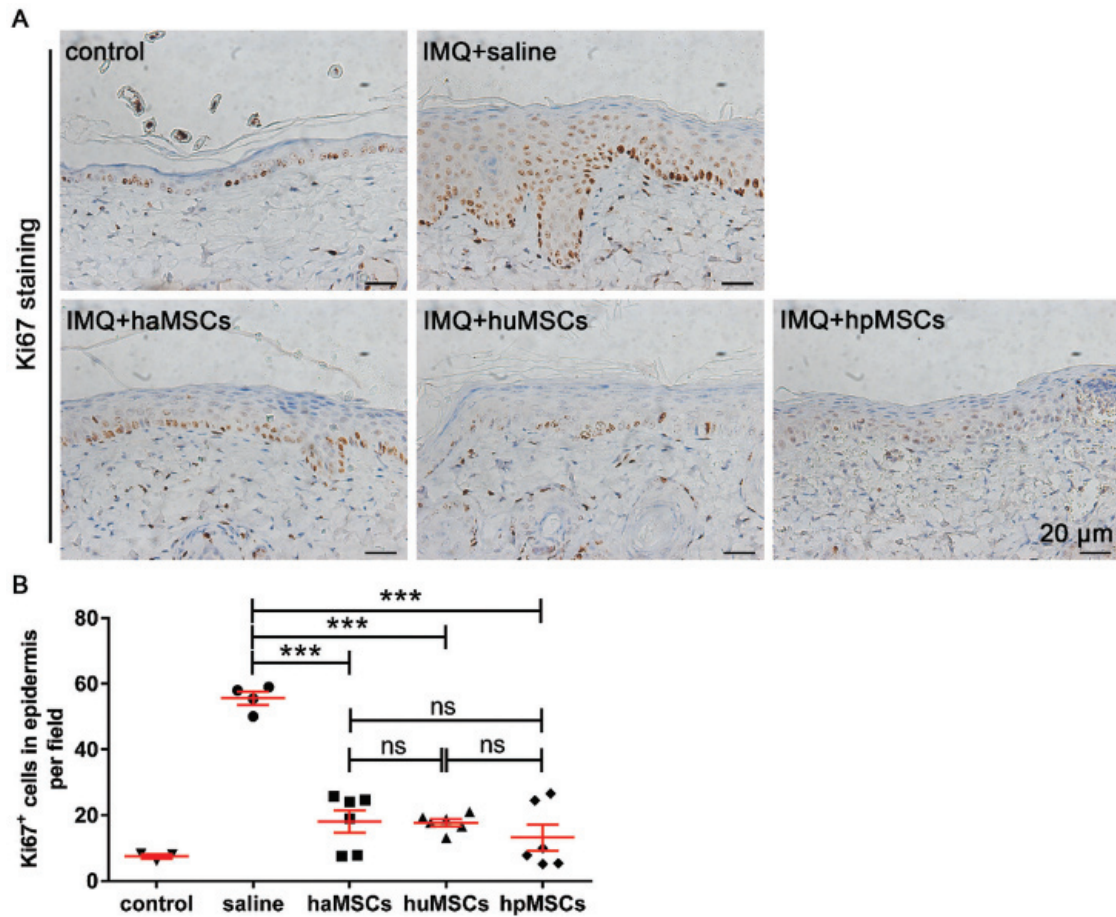
FIGURE 4. Human adipose, placenta, and umbilical cord-derived mesenchymal stem cells decrease proliferation of keratinocytes in the epidermis of IMQ-induced psoriasis mice.
(A) Immunohistochemistry staining of Ki67 in lesional skin of control and IMQ-treated mice. Control mice were not injected. IMQ-treated mice were subcutaneously injected with saline, haMSCs, huMSCs or hpMSCs, respectively. Scale bar: 20 μm. (B) Quantitative analysis of the number of Ki67$^+$ cells per field in the epidermis. ***$p < 0.001$, ns, non-significant, N = 3–6 mice per group.

blood of sheep also exhibit anti-inflammatory activity in the skin wound healing process in a sheep model (Martinello *et al.*, 2018). However, the above tissue source of human MSCs limits their applications for human psoriasis. Human mesenchymal stem cells (MSCs) derived from adipose, placenta, and umbilical cord are easily available and may be extracted in large volume. These characteristics make them ideally suited for a wide spectrum of clinical applications. Thus, we comparatively assessed the effects of haMSCs, hpMSCs, and huMSCs on IMQ-induced psoriasis in mice. The preliminary investigation of our study revealed that the therapeutical effects of these MSCs on the IMQ-induced psoriatic mice model were significant. Our results indicate there is a tendency that the treatment of hpMSCs and huMSCs may be better than that of the haMSCs. However, the statistical differences were not significant among them. As is known to all, haMSCs derived from adipose tissue belong to adult cells, and both hpMSCs and huMSCs, respectively isolated from the placenta and umbilical cord belong to perinatal stem cells. The differentiation potential of the haMSCs may be weak compared with that of the hpMSCs and huMSCs. Therefore, we suggest that it is more beneficial for the treatment of psoriasis using perinatal mesenchymal stem cells.

The previous study has reported that the MSCs could alleviate psoriasis via reducing type I interferon (IFN-I) secreted by plasmacytoid dendritic cells (Chen *et al.*, 2019).

In our study, we found that hyper-proliferation of keratinocytes in the skin of IMQ-induced psoriasis mice could be effectively inhibited by the haMSCs, huMSCs, and hpMSCs, respectively. Thus, the epidermal hyperplasia of IMQ-induced psoriasis mice could be impaired. Our results revealed that it was also impaired that inflammatory infiltration of T lymphocytes to the derma of IMQ-induced psoriasis mice which were individually administrated of haMSCs, huMSCs, and hpMSCs. Subsequently, quantitative real-time PCR analysis showed that the levels of IL-17 expression were markedly suppressed in the lesioned skin of these mice. The previous study has shown that MSCs could decrease the levels of interleukin 6 (IL-6) and transforming growth factor (TGF-β) *in vitro* assay (Darlan *et al.*, 2020). It is inferred that the level of IL-17 produced from Th17 cells could also be reduced by above MSCs treatment *in vivo*. Hence, we conclude that these human MSCs could inhibit the activity of Th17 cells, reducing the level of IL-17 production, which inhibits the proliferation of keratinocytes in lesioned skin of IMQ-induced psoriasis mice. Taken together, IMQ-induced psoriasis mice could be significantly ameliorated by the treatment of haMSCs, huMSCs or hpMSCs through reducing T cell infiltration.

A previous report has shown that the benefits of MSCs therapy could be due to their secretome, which is defined as a
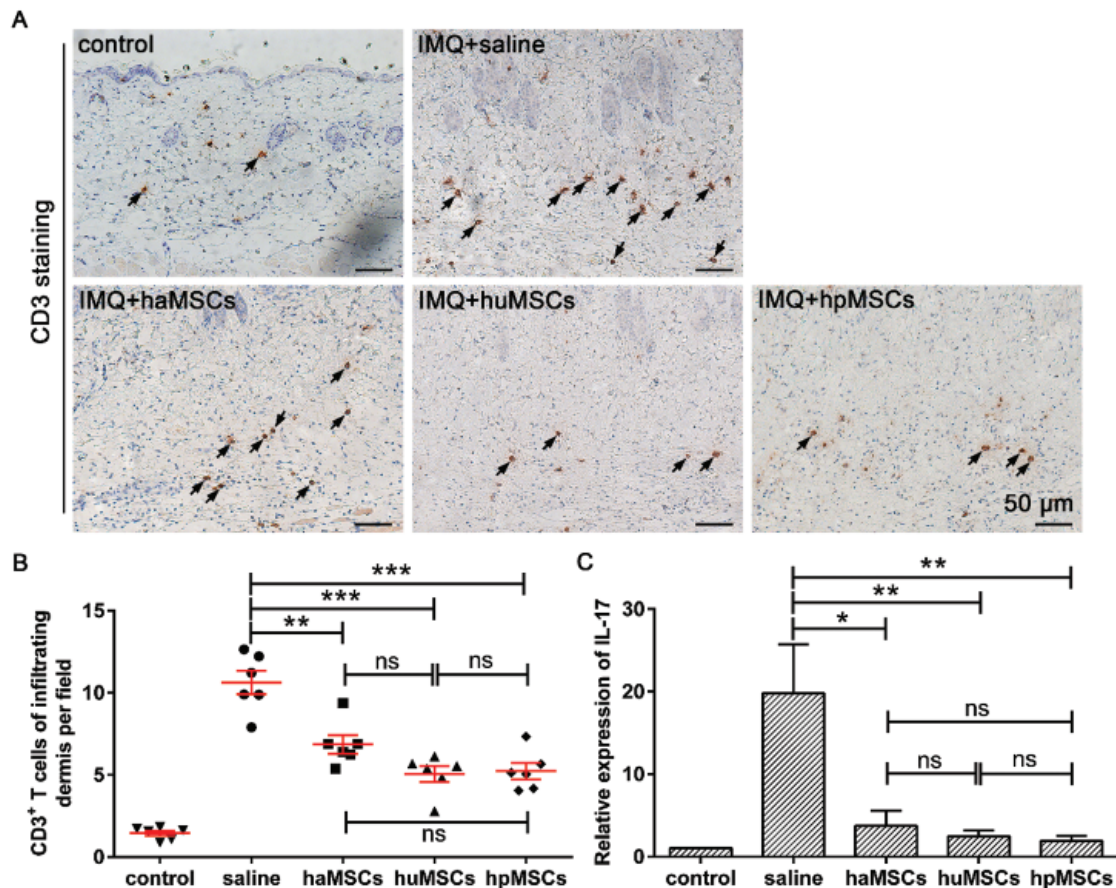
FIGURE 5. Human adipose, placenta, and umbilical cord-derived mesenchymal stem cells lower inflammatory infiltration of T lymphocyte in the derma of IMQ-induced psoriasis mice.

(A) Immunohistochemistry staining of CD3 in lesional skin of control and IMQ-treated mice. Control mice were not injected. IMQ-treated mice were subcutaneously injected with saline, haMSCs, huMSCs or hpMSCs, respectively. Black arrows showed CD3$^+$ T cells in the derma. Scale bar: 50 μm. (B) Quantitative analysis of the number of CD3$^+$ T cells infiltration per field in the derma. $**p < 0.01$, $***p < 0.001$, N = 6 mice per group. (C) Quantitative real-time PCR analysis of the levels of IL-17 expression in lesional skin of mice. $*p < 0.05$, $**p < 0.01$, ns, non-significant, N = 3–6 mice per group.

series of soluble factors and exosomes secreted to the extracellular space (Vizoso et al., 2017). Exosomes may exhibit various advantages over mesenchymal stem cells, including manufacturing, storage, product shelf life, and their potential as a ready-to-go biologic product (Vizoso et al., 2017). Moreover, the application of exosomes could avoid several risks such as tumorigenicity, emboli formation, immune compatibility, and infection transmission (Vizoso et al., 2017). These strengths will be more conducive to the development of cell-free therapy products. Heat shock protein 70 (HSP70) can prevent IMQ-induced psoriasis by regulating T cell activation in mice (Seifarth et al., 2018). Proteomics analysis has revealed that exosomes derived from human MSCs contain various proteins, which include HSP70 in the previous work (Lai et al., 2012). In addition, numerous miRNAs involving the immunomodulatory efficacy were examined by RNA sequencing in exosomal cargo, which includes miR-146 (Song et al., 2017), miR-223 (He et al., 2019), and miR-106b-5p (Shi et al., 2020). Thus, we speculate that human MSCs-derived exosomes maybe contribute to the treatment of psoriasis. However, further studies are needed to clarify whether IMQ-induced psoriasis could be ameliorated by the exosomes derived from the haMSCs, huMSCs, and hpMSCs.

Conclusions

In summary, these data suggest that the treatments of haMSCs, huMSCs or hpMSCs could effectively suppress T lymphocytes recruitment to lesioned skin and reduce the production of IL-17 from Th17 cells in lesioned skin of IMQ-induced psoriasis mice. The results demonstrated that these human MSCs could be applied to the prevention or treatment of IMQ-induced psoriasis in mice. The findings also imply that these human MSCs may be potential therapeutic candidates for skin inflammatory disease or other autoimmune diseases.

Author Contributions: Chengxiang Dai, Suke Li, Meng Li, and Jigang Lei designed the research. Jigang Lei, Zhenyao

Xu, and Zhikai Wang performed the most experiments. Ping Li, Jing Wang, and Yinglu Chen helped with cell isolation and flow cytometry. Xiaole Song, Chengjie Ren, and Meiping Shen performed cell culture and the trilineage differentiation of MSCs. Chengxiang Dai and Jigang Lei analyzed the data. Jigang Lei wrote the manuscript. Chengxiang Dai, Jigang Lei, and Zhikai Wang revised the manuscript. All authors have read and approved the final manuscript.

Conflicts of Interest: Jigang Lei, Suke Li, Meng Li, Ping Li, Jing Wang, Yinglu Chen, Xiaole Song, Chengjie Ren, Meiping Shen, and Chengxiang Dai are current employees of Cellular Biomedicine Group, Ltd. All authors have no conflict of interest.

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