Exosomes Derived from Human Umbilical Cord Mesenchymal Stem Cells Alleviate Psoriasis-like Skin Inflammation

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Abstract

Background

Exosomes are nanovesicles secreted from endosomal membranes. The immunomodulatory effect of mesenchymal stem cells (MSCs) is mediated by MSCs-derived exosomes (MSCs-exo). MSCs-exo are attractive candidates for use in cell therapy for several inflammatory diseases including psoriasis. We investigated whether exosomes derived from human umbilical cord mesenchymal stem cells (hucMSCs-Exo) ameliorate psoriasis-like skin inflammation via the IL-23/IL-17 axis.

Methods

HucMSCs-Exo were isolated by differential ultracentrifugation. Imiquimod (IMQ)-induced mice were subcutaneously injected with hucMSCs-Exo on days 0, 2 and 4. H&E staining and Western blotting were performed, and tissue histopathology and STAT3/p-STAT3, IL-17, IL-23, and CCL20 levels were assessed. HucMSCs-Exo were co-cultured with dendritic cells (DCs) and HaCat cells in vitro. Western blotting, flow cytometry and ELISAs were then performed to measure STAT3/p-STAT3, IL-17, IL-23, and CCL20 levels.

Results

Subcutaneous injection of hucMSCs-Exo significantly ameliorated symptoms and diminished the clinical and pathological scores of psoriasis in IMQ-induced mice. In MSCs-exo-treated mice, STAT3/p-STAT3, IL-17, IL-23 and CCL20 levels were decreased. HucMSCs-Exo co-cultured in vitro with DCs suppressed DC maturation and activation and inhibited IL-23 secretion. HucMSCs-Exo co-cultured with HaCat cells reduced STAT3/p-STAT3, IL-17, IL-23, and CCL20 levels.

Conclusions

HucMSCs-Exo can effectively ameliorate psoriasis-like skin inflammation in mice via the IL-23/IL-17 axis. Furthermore, hucMSCs-Exo exhibit immunomodulatory potency by inhibiting the maturation and activation of DCs and blocking the positive feedback effect of IL-17 on keratinocytes. Our data offer a novel therapeutic approach for chronic inflammatory skin diseases such as psoriasis by leveraging the immunomodulatory effects of hucMSCs-Exo.

1 Introduction
Mesenchymal stem cells (MSCs), the major stem cells in cell therapy, have been used in the clinic for more than 10 years and have proven to be effective for the treatment of various intractable autoimmune and inflammatory disorders because of their distinct immunomodulatory properties [1–4]. MSCs inhibit CD4+ T cell proliferation and differentiation, dendritic cells (DCs) maturation and human Th17 cell differentiation and function [5–7]. In addition, MSCs reduce inflammatory cytokine (tumour necrosis factor-α (TNF-α), interleukin (IL)-1, and IL-6) levels [8] and inhibit NF-κB activation under inflammatory conditions [9]. A previous preclinical study demonstrated that allogeneic MSC therapy has a beneficial therapeutic effect in psoriasis [10–12]. However, MSC therapy has several disadvantages, such as the difficulty in quality control before administration, the short half-life and nonspecific differentiation of MSCs, the potential for tumour formation, poor engraftment efficiency and unwanted immune responses [13]. Recent studies have shown that the majority of the therapeutic benefits of MSCs result from exosomes [14–16].

Exosomes are nanovesicles (50–200 nm) derived from multivesicular bodies and released by most living cells and are considered to play an important role in transmitting information between cells [17]. Recent studies suggest that MSC-derived exosomes (MSCs-exo) recapitulate the therapeutic efficacy of MSCs, including their anti-inflammatory effects [18–19] and do not have most of the disadvantages associated with MSC therapy; for example, MSCs-exo are easier to store at – 20 °C and are standardized [20], carry no risk of tumour formation, circulate easily throughout the body and reach sites of injury, and do not elicit toxicity [21]. Previous studies have applied MSCs-exo to treat spinal cord injury [22], liver fibrosis [13], and graft versus host disease (GVHD) [23], and they achieved good curative effects. In our current study, we demonstrated that exosomes derived from human umbilical cord MSCs (hucMSCs-Exo) prevented the progression and reduced the severity of psoriasis by regulating immune cells, such as DCs and Th17 cells, along with HaCat cells.

Psoriasis is a chronic immune-mediated inflammatory skin disease that affects over 125 million people worldwide, not only physically but also socially, emotionally and financially [24]. Its histological features are characterized by epidermal hyperplasia, increased angiogenesis and immune cell infiltration. Although the pathogenesis of psoriasis is not fully understood, IL-23 secreted by DCs
and monocytes/macrophages is recognized to promote the expansion of IL-17–producing T cells, which exert pleiotropic effects on the recruitment and activation of other immune cells such as neutrophils as well as on keratinocyte (KC) hyperplasia [25]. The recent therapeutic success of anti-IL-23 and anti-IL-17 antibodies has shown that the IL-23/IL-17 cytokine axis plays a pivotal role in the inflammatory response in psoriasis, confirming that the IL-23/IL-17 axis is a good target for psoriasis treatment.

In our study, we investigated the therapeutic effects of hucMSCs-Exo in a mouse model of imiquimod (IMQ)-induced psoriasis and the potential effect of hucMSCs-Exo on DCs and HaCat cells. Treatment with hucMSCs-Exo efficiently blocked the induction of inflammatory cytokines and reduced both histopathological symptoms and immune responses in these animals.

2 Materials And Methods

2.1 Animals

Female C57/BL6 mice (8 weeks) were provided by the Animal Institute of Shandong University and housed individually in separate cages with free access to water and food in a room with an alternating 12-h light/dark cycle and controlled temperature (22–25 ºC) and humidity (40–60%). Female C57/BL6 mice (4–6 weeks) were used for DC isolation.

2.2 Cell culture and exosome extraction

MSCs is kindly donated by Central Laboratory of Liaocheng People’s Hospital. HucMSCs at passage 4 were cultured in exosome-free medium for 48 h. The conditioned medium was collected (approximately 60 ml), centrifuged at 300× for 10 minutes and 16,500× for 30 minutes, and filtered through a 0.22 μm pore size filter to remove cell debris. At each of these steps, the pellet was discarded, and the supernatant was used for the following step. The final supernatant was then ultracentrifuged at 100,000 ×g for 70 minutes to pellet the small vesicles corresponding to exosomes. The pellet was filtered through a 0.22 μm pore size filter to eliminate contaminating proteins and centrifuged one final time at the same high speed. The purified exosome pellets were resuspended in PBS and prepared for identification. Finally, the exosomes were filtered through a 0.22 μm pore size filter for functional experiments.
2.3 Characterization of hucMSCs-Exo

The morphologic characteristics of hucMSCs-Exo were observed by transmission electron microscopy (TEM). The number of hucMSCs-Exo was quantified using nanoparticle tracking analysis. The phenotypic profile of hucMSCs-Exo was determined by Western blotting with antibodies specific for CD9, CD63 and CD81. The protein content of hucMSCs-Exo was quantified with a BCA Protein Assay kit.

2.4 In vivo experiments

2.4.1 Animal model

All mice were randomly divided into 4 groups (n= 6 mice/group): control group (Normal), IMQ group (IMQ), PBS-treated IMQ group (PBS + IMQ), and hucMSCs-Exo-treated IMQ group (hucMSCs-Exo + IMQ). All experimental procedures were conducted in accordance with the Animal Use and Care Committee of Shandong University. To establish the IMQ model, the C57BL/6 mice were treated with a daily topical dose (62.5 mg) of 5% IMQ cream (Aldara, 3M Health Care Limited) on the shaved dorsal skin for 6 days. On days 0, 2 and 4 (Fig. 2(A)), the mice in the hucMSCs-Exo + IMQ group were subcutaneously injected with exosomes (50 µg/mouse), and the mice in the PBS + IMQ group were subcutaneously injected with PBS (50 µl/mouse). All mice were euthanized on day 7 for analysis. The dorsal skin of the mice was sectioned, and part of the skin was stained with haematoxylin and eosin (H&E) for histological evaluation. Another part was used for Western blotting.

2.4.2 Histological evaluation

Skin sections from the dorsal surface of the mice were stained with H&E for histological evaluation. Psoriasiform epidermal hyperplasia was assessed microscopically in the sections as reported earlier [26–27].

2.4.3 Mouse skin tissue test

We collected skin tissue from the dorsal surface of each group of mice and used Western blotting to detect STAT3/p-STAT3, IL-17, IL-23, and CCL20, with GAPDH as the internal control.

2.5 In vitro experiments

2.5.1 Generation and differentiation of bone marrow-derived DCs (BM-DCs) and hucMSCs-Exo co-
cultures

We observed whether hucMSCs-Exo affected the activation and maturation of DCs and the release of cytokines. DCs were obtained from the BM of normal 4- to 6-week-old female C57BL/6 mice according to a previously described protocol [28]. BM cell pellets were resuspended in RPMI-1640 medium (Gibco) supplemented with 10% FBS (Gibco, Australia), 1% penicillin-streptomycin, 20 ng/ml rmGM-CSF (PeproTech, USA), and 20 ng/ml rmIL-4 (PeproTech, USA) at $1 \times 10^6$ cells/ml and transferred into a 6-well plate. On day 3 of culture, non-adherent cells were gently removed, and fresh culture medium was added. Fresh culture medium was added every 2 days thereafter to induce DC differentiation. For DC maturation, on day 7, non-adherent cells were collected, and DCs were washed, resuspended at $2 \times 10^5$ cells/ml in culture medium with 10 ng/ml rmTNF-α (PeproTech, USA), and seeded in a 24-well plate, with or without hucMSCs-Exo (2.5 µg/ml) co-cultured for another 24 h. The different groups of cells were collected to detect the phenotype of DCs by flow cytometry, and the cell supernatants were collected and centrifuged at $1000 \times g$ for 20 minutes at 4 °C. Then, the supernatants were stored at −80 °C and utilized for IL-23p40 ELISAs within one month.

2.5.2 HaCat cell and hucMSCs-Exo co-cultures

We aimed to observe whether hucMSCs-Exo affect inflammatory factors and chemokines in keratinocytes. HaCat cells (Zhongqiao Xinzhou, China) were used as the cell model system. When the cells had grown to nearly 90% confluency, they were seeded in 6-well flat-bottom plates ($2 \times 10^5$ cells/ml). When the cells had grown to nearly 80% confluency, they were starved overnight in serum-free and antibiotic-free medium and were then stimulated with 100 ng/ml IL-17A (PeproTech, USA) for the indicated times with or without hucMSCs-Exo (2.5 µg/ml) for 24 h. The different groups of HaCat cells were harvested, and cellular proteins were extracted for Western blotting to detect STAT3/p-STAT3 and IL-17, with GAPDH as the internal control. The cell supernatants were collected and centrifuged at $1000 \times g$ for 20 minutes at 4 °C. Then, the supernatants were stored at −80 °C and utilized for IL-23 and CCL20 ELISAs within one month.

2.6. Western blot analysis
The different groups of HaCat cells and mouse skin tissue were harvested and lysed in modified RIPA buffer with combined protease and phosphatase inhibitors. Protein samples (30 µg/lane) were separated by 12% SDS-PAGE and transferred to PVDF membranes (Millipore, USA). Membranes were blocked with 5% BSA in TBST for 2 h at room temperature (RT) and incubated with primary rabbit monoclonal antibodies specific for CD9 (1:2000, Abcam, England), CD63 (1:1000, Abcam, England), CD81 (1:1000, Abcam, England), GAPDH (1:1000, Abcam, England), STAT3 (1:1000, Abcam, England), p-STAT3 (1:1000, Abcam, England), IL-17A (2 µg/ml, Abcam, England), IL-23 (1 µg/ml, Abcam, England), and CCL20 (0.2 µg/ml, Abcam, England) at 4 °C overnight. The next day, membranes were incubated with secondary antibodies for 1 h at room temperature and visualized using chemiluminescence. The data were analysed with imaging software.

2.7 Measurement of inflammatory cytokines by ELISA

Supernatants of in vitro cell cultures were analysed via ELISA using a commercially available ELISA kit. The protein level of IL-23p40 (Elabscience, China) was evaluated in the DC supernatant. The protein levels of IL-23p19 (Elabscience, China) and CCL20 (Elabscience, China) were evaluated in the HaCat cells supernatant. The absorbance at 450 nm was measured using an ELISA plate reader (Bio-Rad, USA).

2.8 Flow cytometric analysis

To analyse the DC phenotype, cytofluorimetric analysis of DCs was performed by triple-colour staining. Cells were incubated with the following fluorochrome-conjugated antibodies for 30 minutes at 4 °C: FITC-anti-mouse CD11c (N418, hamster IgG, eBioscience, USA), APC-anti-mouse CD86 (GL1, rat IgG2a, eBioscience, USA), and PE-anti-mouse MHCII (M5/114.15.2, rat IgG2b, eBioscience, USA). Then, cells were centrifuged at 300 × g for 5 minutes at room temperature, washed, resuspended in PBS, and analysed in a FACSCalibur equipped with Cell Quest software (BD Biosciences).

2.9 Statistical analysis

Data are presented as the means plus or minus the SD. All experiments were performed at least three times. Statistical analysis was performed using GraphPad Prism 7.00 (GraphPad Software, La Jolla, CA, USA.). P values of < 0.05 were considered statistically significant, and the level of significance is
indicated as follows: *, P < 0.05 and **, P < 0.01.

3 Results

3.1 Characterization of hucMSCs-Exo

TEM of hucMSCs-Exo samples showed the presence of numerous cup-shaped vesicles with diameters of 50–200 nm (Fig. 1(A)). HucMSCs-Exo expressed high levels of CD9, CD63, and CD81, although no expression of CD9, CD63, or CD81 was detected in HucMSCs (Fig. 1(B)). The exosomes were visualized and the diameter distribution was calculated via nanoparticle tracking analysis (Fig. 1(C)). These results verified that we successfully isolated exosomes from hucMSCs.

3.2 HucMSCs-Exo prevent the development and ameliorate the severity of IMQ-induced psoriasis in mice by suppressing the secretion of cytokines and chemokines

3.2.1 HucMSCs-Exo ameliorate the severity of psoriasis-like skin inflammation in mice

Phenotypically, both IMQ-treated groups started to display signs of erythema, scaling, and thickening, followed by inflammation, which continuously increased in severity until the end of the experiment. H&E staining showed an increase in the epidermal thickness (Fig. 2(B)) and mononuclear cell infiltration in the IMQ group (Fig. 2(C, D)). These findings showed that we successfully established the model.

Compared with the IMQ + PBS group, the IMQ + hucMSCs-Exo group exhibited highly reduced psoriatic erythema, scaling, and thickening (Fig. 2(B)). In addition, the Psoriasis Area and Severity Index (PASI) scores of mice in the IMQ + hucMSCs-Exo group were significantly lower than those of mice in the PBS group (Fig. 2(E)), and H&E staining showed decreases in the epidermal thickness (Fig. 2(C,D,F)) and mononuclear cells infiltration (Fig. 2(C,D)). These data showed that hucMSCs-Exo ameliorated IMQ-induced psoriasis-like skin symptoms in mice.

3.2.2 HucMSCs-Exo inhibit signalling pathways and the expression of inflammatory factors and chemokines

Subsequently, we studied the effect of hucMSCs-Exo on the expression of inflammatory mediators in the skin of psoriatic mice. Western blot analysis showed that compared with the control groups, the IMQ and PBS + IMQ groups exhibited significantly elevated protein levels of STAT3/p-STAT3, IL-17, IL-
However, subcutaneous injection of hucMSCs-Exo effectively reduced the protein levels of STAT3/p-STAT3, IL-17, IL-23, and CCL20 (Fig. 3), consistent with the observed histological changes. These results implied that hucMSCs-Exo inhibited psoriasis-like skin inflammation through inhibiting the levels of STAT3/p-STAT3, IL-17, IL-23, and CCL20.

3.3 HucMSCs-Exo affect both DCs and keratinocytes

3.3.1 HucMSCs-Exo co-cultured with DCs inhibit the maturation and activation of BM–DCs, and the secretion of IL-23

The percentage of cells gated for analysis of CD11c+ expression was greater than 70% in all samples and did not differ significantly across the samples (Fig. 4(A)). The surface molecule phenotypes of immature DCs (imDC) showed mean percentages of CD11c+MHCIi+ and CD11c+CD86+ of 55.43%±10.94% and 53.46%±7.49% (Fig. 4(B)), respectively; in parallel, the mean percentages of CD11c+MHCIi+ (73.55%±3.89%) and CD11c+CD86+ (59.20%±7.47%) mDCs were upregulated in the absence of hucMSCs-Exo (Fig. 4(B)). In contrast, in the presence of hucMSCs-Exo, the mean percentages of CD11c+MHCIi+ (65.14%±8.39%) and CD11c+CD86+ (50.73%±5.20%) were downregulated (Fig. 4(A, B)). These findings indicate that MSCs exert a strong inhibitory effect on the maturation and activation of DCs.

Next, we detected cytokine production by DCs upon hucMSCs-Exo treatment. The expression of IL-23p40 exhibited a decreasing trend. These results indicated that hucMSCs-Exo not only inhibit the maturation and activation of DCs but also decrease the production of IL-23p40 (Fig. 4(c)).

3.3.2 HucMSCs-Exo suppress HaCat cells secretion of cytokine and chemokines by inhibiting STAT3 activity

In this study, we investigated the role of hucMSCs-Exo in the IL-23/IL-17 axis signalling pathway. HaCat cells were treated with IL-17A and hucMSCs-Exo to assess their effect on cytokine and chemokine secretion. HucMSCs-Exo inhibited the secretion of cytokines and chemokines such as IL-23 (Fig. 5(C)) and CCL20 (Fig. 5(C)) and reduced the protein level of IL-17 (Fig. 5(A, B)). This result was
consistent with those of our in vivo experiments.

To further examine the mechanisms underlying the reduction in inflammatory responses by hucMSCs-Exo, we measured STAT3 and p-STAT3 levels in IL-17-stimulated HaCat cells. HucMSCs-Exo significantly reduced the protein expression levels of STAT3 and p-STAT3 (Fig. 5(A, B)). These data supported the hypothesis that the immune mediator STAT3 is strongly antagonized by hucMSCs-Exo in psoriasis.

4 Discussion

HucMSCs are attractive immune modulators because they act on various immune and non-immune cells. The therapeutic effects of hucMSCs on psoriasis in experimental studies and clinical cases have previously been reported [10–11], In clinical two cases of psoriasis vulgaris treated with hucMSCs, both of the them maintained relapse-free for four or five years. The immunomodulatory properties of hucMSCs may indirectly mediated through the exosomes, which have been considered as a promising candidate for a novel cell-free therapy. MSCs-exo has therapeutic effects in various relapsing inflammatory disorders, such as inflammatory bowel disease, atopic dermatitis and chronic GVHD (cGVHD) [23, 29–30]. Accumulative evidences have indicated that MSCs-exo exhibited potent immunomodulatory effects by regulating the activation of immune cells and inhibiting the expression of various inflammatory cytokines.

Psoriasis is driven by interactions between activated immunocytes (such as DCs) and keratinocytes. Recently, a new theory regarding the role of the IL-23/IL-17 axis in psoriasis has become increasingly validated [31–33]. Mature DCs (mDCs) can produce IL-23, which can stimulate Th17 cell activation and proliferation, with the consequent release of inflammatory cytokines such as IL-17. IL-17 acts on keratinocytes, leading to epidermal hyperplasia, acanthosis, and hyperparakeratosis, which are key characteristics of psoriasis. In this study, we found that hucMSCs-Exo significantly alleviated the severity of psoriasis, and the skin erythema, scaling and epidermal thickness was obviously decreased. Our results demonstrated that subcutaneously injected hucMSCs-Exo significantly improved clinical symptoms and inflammatory infiltration in IMQ-induced psoriatic mice.

DCs play a pivotal role in the pathogenesis of psoriasis. Based on a previous study, MSCs inhibited
early DC maturation [6], and either MSCs or MSC-derived extracellular vesicles (MSC-EVs) co-culture with DCs downregulated the secretion of IL-23 and IL-22 [34]. Our results showed that HucMSCs-Exo inhibited the secretion of IL-23 by inhibiting the maturation and activation of DCs. In addition, treatment with hucMSCs-Exo decreased the expression of IL-23 in the skin of IMQ-induced psoriatic mice, which consistent with our in vitro experiment results. In brief, our studied indicated that the mechanism of hucMSCs-Exo ameliorated psoriasis-like skin inflammation might though inhibiting the functions of DCs and the secretion of IL-23.

More and more evidence showed that there is an overactive Th17 response in psoriasis-like inflammation [35]. Activated keratinocytes play an important role in the inflammatory environment in psoriasis though producing IL-23, which can mediate cross-talk with Th17 lymphocytes in synergy with IL-23 secreted by mDCs. Moreover, keratinocytes also secrete chemokines such as CCL20 that efficiently recruit Th17 cells into the lesions. In this study, we found that hucMSCs-Exo inhibited the expression of IL-17 in HaCat cells and the secretion of IL-23 and CCL20 by HaCat cells. Simultaneously we further found that hucMSCs-Exo inhibited the expression of the IL-17/IL-23/CCL20 in the skin of IMQ-induced psoriatic mice. Our results demonstrated hucMSCs-Exo could block the positive feedback effect of IL-17 on keratinocytes directly.

As a central regulator of inflammatory and immune responses, Signal Transducer and Activator of Transcription 3 (STAT3) mediates the IL-23/IL-17 axis which is involved in the pathogenesis of psoriasis. Activated STAT3 is closely related to Th17 cell differentiation and the keratinocytes' response to inflammation [36]. Cui et al reported that exosomes derived from both MSCs and hypoxic MSCs prevented STAT3 activation [37]. In our experiments, we found that hucMSCs-Exo inhibited the expression of STAT3/p-STAT3 in the epidermis of IMQ-induced psoriatic mice and HaCat cells, which suggested that hucMSCs-Exo downregulated the expression of key molecules in the IL-23/IL-17 axis probably by inhibiting STAT3/p-STAT3.

The composition of hucMSCs-Exo is complex, and the specific component of hucMSCs-Exo which contributes to the biological activities is unknown. Indeed MSC-EVs contain various MSC-derived proteins, lipids, DNA, mRNAs, and miRNAs; among them, miRNAs have received the most attention.
because they play an important role in regulating gene expression. In one study, in another report, exosomes from PC-MSCs could improve the learning and memory capabilities of APP/PS1 mice via regulation of inflammatory responses by regulating miR-21[37]. miR-181c in EVs was found to be critical for immunoregulation that efficiently reduced inflammation in burned rats [38]. Li et al. found that MSCs-Exo reversed the increased Th17/Treg in AA through SphK1-mediated exosomal S1P enrichment [39]. Since precise mechanisms of action or key therapeutic factors have not been disclosed, we hypothesize that the miRNAs contained in hucMSCs-Exo may play a key role in immune regulation. Next step, we plan to perform a comparative transcriptome/proteomic analysis of hucMSCs-Exo to reveal the mechanism underlying the role of hucMSCs-Exo in psoriasis.  

Conclusion  
In conclusion, our findings suggested that exosomes from hucMSCs inhibited the expression of IL-23 in DCs and alleviated inflammatory responses. Moreover, exosomes directly blocked the positive feedback effect of IL-17 on keratinocytes, which attenuated psoriasis-like inflammation. Our results suggested that hucMSCs-Exo may be a promising agent for the treatment of psoriasis.  

Abbreviations  
MSCs: mesenchymal stem cells; MSCs-exo: MSCs-derived exosomes; hucMSCs-Exo: exosomes derived from human umbilical cord mesenchymal stem cells; IMQ: Imiquimod; DCs: dendritic cells; TNF-α: tumour necrosis factor-α; IL: interleukin; GVHD: graft versus host disease; TEM: transmission electron microscopy; BM: bone marrow; MSC-EVs: MSC-derived extracellular vesicles  

Declarations  

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Authors’ contributions  
SQ provided the research conception and design. YJ and LJ collaborated and guided the experimental operations. ZL performed the experiments and acquired, analyzed, and interpreted the data and drafted this manuscript. All authors reviewed and approved the final manuscript.  

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Availability of data and materials

All data generated or analysed during this study are included in this published article.

Ethics approval and consent to participate

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Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflicts of interest to report.

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Figures

Characterization of hucMSCs-Exo. a. Morphology of hucMSCs-Exo under a transmission electron microscope. (Scale bar, 500 nm.) b. CD9, CD63, and CD81 expression in exosomes was detected by Western blotting. c. Nanoparticle tracking analysis of exosomes.
HucMSCs-Exo inhibit epidermal hyperproliferation, decrease acanthosis and prevent the development and reduce the severity of IMQ-induced psoriasis in mice. a. The experimental plan. b. Dorsal skin of normal control mice, IMQ-induced mice, IMQ-induced mice subcutaneously injected with PBS, and IMQ-induced mice subcutaneously injected with hucMSCs-Exo. Photographs were taken on the seventh day after application of IMQ for 6 consecutive days. c, d. H&E staining of the dorsal skin of mice from the four groups. c: Original magnification, 200 ×, scale bar, 50 μm, d: Original magnification, 400 ×, scale bar, 20 μm. e. PASI scores of mouse lesional skin. The cumulative score (erythema + scaling + thickness) was calculated. The data are presented as the means ± SDs. n = 6 (**P<0.01). f. Measurement of the epidermal thickness in mice from the four groups. (The data are presented as the means ± SDs. HucMSCs-Exo-treated group vs. IMQ+PBS-treated group in all data. **P<0.01).
Figure 3

HucMSCs-Exo ameliorate IMQ-induced psoriasis-like skin inflammation in mice. a. We collected the dorsal skin of mice in the control, IMQ, PBS+IMQ and hucMSCs-Exo+IMQ groups and extracted the protein from mouse skin tissue for Western blot analysis to evaluate IL-23, IL-17, and CCL20 expression. Western blot analysis was performed with antibodies specific for the molecules indicated. GAPDH was used as the control. b. The pixel densities of the bands were divided by those of the corresponding GAPDH bands for normalization. The data are presented as the means ± SDs (*P < 0.05, **P<0.01).
HucMSCs-Exo exert a suppressive effect on DC maturation and activation and decrease IL-23 secretion by DCs. a and b. HucMSCs-Exo affected the maturation and activation of DCs.

The results shown are representative of three independent experiments. The data are presented as the means ± SDs (*P < 0.05). c. HucMSCs-Exo inhibited the production of IL-23p40. The data are presented as the means ± SDs (*P < 0.05).
HucMSCs-Exo inhibit cytokine and chemokine secretion after IL-17A stimulation. a. Protein levels of STAT3/p-STAT3 and IL-17 in hacat cells as visualized by Western blotting. GAPDH was used as the internal control. b. The pixel densities of the bands were divided by those of the corresponding GAPDH bands for normalization. The data are presented as the means ± SDs (**P<0.01). c. The levels of IL-23 and CCL20 in cell culture supernatants were measured by ELISA kits. The data are presented as the means ± SDs (**P<0.01).