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Research article

Human dental pulp stem cells ameliorate the imiquimod-induced psoriasis in mice

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ABSTRACT

Psoriasis is an autoimmune disease, which has a significant impact on the quality of patient's life. And, there is still no cure for psoriasis. The human dental pulp stem cell (hDPSC) possesses the properties of immunoregulation. In this study, we aimed to determine the effect of hDPSC on the imiquimod (IMQ)-induced psoriasis in mice. The psoriasis model was established by topical application of IMQ cream in mice for 7 days. We found that subcutaneous injection of hDPSC could reduce the symptoms of skin lesions in IMQ-induced psoriasis and suppress the expression of keratin 16, S100A8, S100A9, which are associated with abnormal epidermal proliferation. Subepithelial inflammatory cytokines, $CD4^+$ T lymphocytes and $CD11c^+$ dendritic cells infiltrations were significantly inhibited in by hDPSC. The TNF-α, IFN-γ expressions in serum were decreased, and splenomegaly induced by IMQ was improved after hDPSC treatment. In summary, our study demonstrated that hDPSC could reduce the symptoms of skin lesions and suppress local and systemic immune responses of IMQ-induced psoriasis in mice, which might provide a new sight for the treatment of psoriasis.

1. Introduction

Psoriasis is a common chronic autoimmune skin disease associated with heredity. Some studies have reported that the incidence of psoriasis is significantly higher in first- and second-degree relatives than in the general population [1], among which HLA-Cw6 and CARD14 are important genetic factors associated with psoriasis [2]. The worldwide prevalence is 2%–3%, and is increasing year by year [3]. The main clinical manifestations are erythema, silvery scale and epidermal thickening in skin lesions [4]. The histological manifestations are abnormal epidermal hyperplasia, parakeratosis, increased angiogenesis, inflammatory factors and immune cells infiltration [5]. Although the pathogenesis of psoriasis is not fully elucidated, numerous evidences indicate that T cells, especially helper T cells (Th cells), play an important pathogenic role in the initiation of psoriasis [6,7]. Increasing Th1 cytokines (tumor necrosis

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factor α, TNF-α; interferon γ, IFN-γ) and Th17 cytokines (interleukin 17, IL-17; interleukin 23, IL-23) can be detected in the skin and peripheral blood [8]. Psoriasis is not only limited to the skin, but also affects joints and other organs complicating multisystem diseases such as metabolic disorders and cardiovascular diseases [9]. At present, the treatments for psoriasis are mainly topical agents, such as topical corticosteroids [10], sunitinib [11] and calcipotriol [12]. But none of these treatments for psoriasis is considered curable, and requires long-term topical application. The relapsing-remitting nature of psoriasis has a great adverse impact on patient's life. Therefore, the research of effective treatment for psoriasis is urgently needed.

hDPSC is isolated from pulp tissues of permanent teeth (usually impacted teeth) and deciduous teeth. It is easy to isolate, abundant in sources and has less ethical concerns [13]. hDPSC originating from neural crest has the capability of multi-lineage differentiation, which can differentiate into osteoblasts, chondrocytes, odontoblasts, adipocytes and neural-like cells [14]. Therefore, hDPSC has gathered much attention. Recently, many studies have found that hDPSC possesses immunomodulatory properties [15,16]. They exert immunomodulatory effects on natural killer (NK) cells, dendritic (DC) cells, T lymphoid cells and B lymphoid cells by interacting with the innate and acquired immune systems [17]. In addition, hDPSC can release soluble immunosuppressive molecules such as prostaglandin E2 (PGE2), transforming growth factor β (TGF-β), and nitric oxide (NO) in a paracrine manner to suppress the T cell inflammatory response [18]. Studies have reported that hDPSC is able to treat a variety of autoimmune and inflammation-related diseases in vivo and vitro [19,20]. However, studies on the treatment of psoriasis by hDPSC have not been reported.

In this study, we investigated the therapeutic effect of hDPSC on IMQ-induced psoriasis in mice, and provided experimental evidence for its clinical application.

2. Materials and methods

2.1. Isolation and culture of hDPSC

hDPSC was isolated from the impacted teeth of healthy adults aged 18–22 years with informed consent. These impacted teeth were derived from the third molars that have no chewing function in the mouth. And they might cause recurrent pericoronitis, caries in adjacent teeth, or needed to be extracted for orthodontic treatment. Approval was obtained from the Ethics Committee of Nanjing Stomatological Hospital, Medical school of Nanjing University (NJSH-2022NL-36). The primary hDPSC was isolated and cultured as previously described [21]. Briefly, pulp tissues were extracted from the dental pulp cavity, cut into tiny pieces (<1 mm^3), and digested with 3 mg/mL collagenase type I (Roche, Switzerland) for 1 h at 37 °C. Cells were cultured and expanded in Dulbecco's modified Eagle's medium (DMEM; Gibco, USA) with 10% fetal bovine serum (FBS; Gibco, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin (Gibco, USA) at 37 °C and 5% CO_2. The third passage hDPSC was used in all experiments.

2.2. Identification of hDPSC

Osteogenic differentiation assay was performed as previously described [22]. The third passage hDPSC was cultured in mineralization induction medium (DMEM supplemented with 50 mg/mL ascorbic acid, 10 mM sodium β-glycerophosphate and 10 nM dexamethasone). Medium was changed every 3 days. After 2 weeks, cells were fixed with 4% paraformaldehyde and stained with Alkaline Phosphatase (ALP; Beyotime, China) and Alizarin Red S (BestBio, China) to detect the ALP activity and mineralized nodules.

Adipogenic differentiation assay was performed as previously described [23]. The third passage hDPSC was cultured with adipogenic induction medium (DMEM supplemented with 0.5 mM isobutyl-methylxanthine, 1 mM dexamethasone, 10 mM insulin, 200 mM indomethacin, 50 mg/mL of gentamicin) for 2 weeks. Medium was changed every 3 days. Afterwards, cells were stained with oil red O (OriCell, China) to detect the presence of lipid droplets.

The surface antigen markers of hDPSC were detected by flow cytometry. As previously described [24], the third passage hDPSC was washed, resuspended and incubated in PBS (Gibco, USA) containing 3% FBS for 45 min with primary antibodies against CD45 (Biolegend, USA), CD29 (Biolegend, USA), CD73 (Biolegend, USA) and CD90 (Biolegend, USA). Flow cytometry analysis was performed by Flow cytometer (BD FACSVerse, USA) and FlowJo software.

2.3. Mice and treatments

Female BALB/c (7-week-old) mice, initial body weight about 20 g, were purchased from China Pharmaceutical University. The qualification certificate number of animal caretaker is 220181305. Ethical approval was obtained from Animal Ethical and Welfare Committee of Nanjing University (IACUC-D2102033). These mice were adapted for a week before the experiment began. One day before the experiment (day 0), the back hair of BALB/c mice was shaved by electric shaver to form an exposed area of 2 cm × 3 cm. They were then divided into four groups ($n = 5$/group): Control group, IMQ-induced group (IMQ), Calcipotriol (Cap)-treated group (IMQ + Cap) and hDPSC treated-group (IMQ + hDPSC). The mice in IMQ, IMQ + Cap and IMQ + hDPSC groups received a topical application of 62.5 mg IMQ cream (Med Shine pharmaceutical, China) on the shaved back skin at 10 a.m. every day for 7 days to induce psoriasis-like skin symptoms. During the consecutive application of IMQ cream for 7 days, mice in IMQ + hDPSC group were subcutaneously injected with the third passage hDPSC on day 3 and day 5, respectively (2.5×10^6 cells/mice in 200 μL PBS [25]). And mice in IMQ + Cap group were topically treated with Cap on day 3 and day 5, respectively. Mice in the Control group received 62.5 mg vehicle cream (Vaseline; Med Shine pharmaceutical, China) on the shaved back skin at 10 a.m. every day for 7 days. Control and IMQ groups were subcutaneously injected with 200 μL PBS on day 3 and day 5. All mice were sacrificed on day 7 and their dorsal skin, blood serum and spleen were collected for subsequent analysis (Fig. 2A).

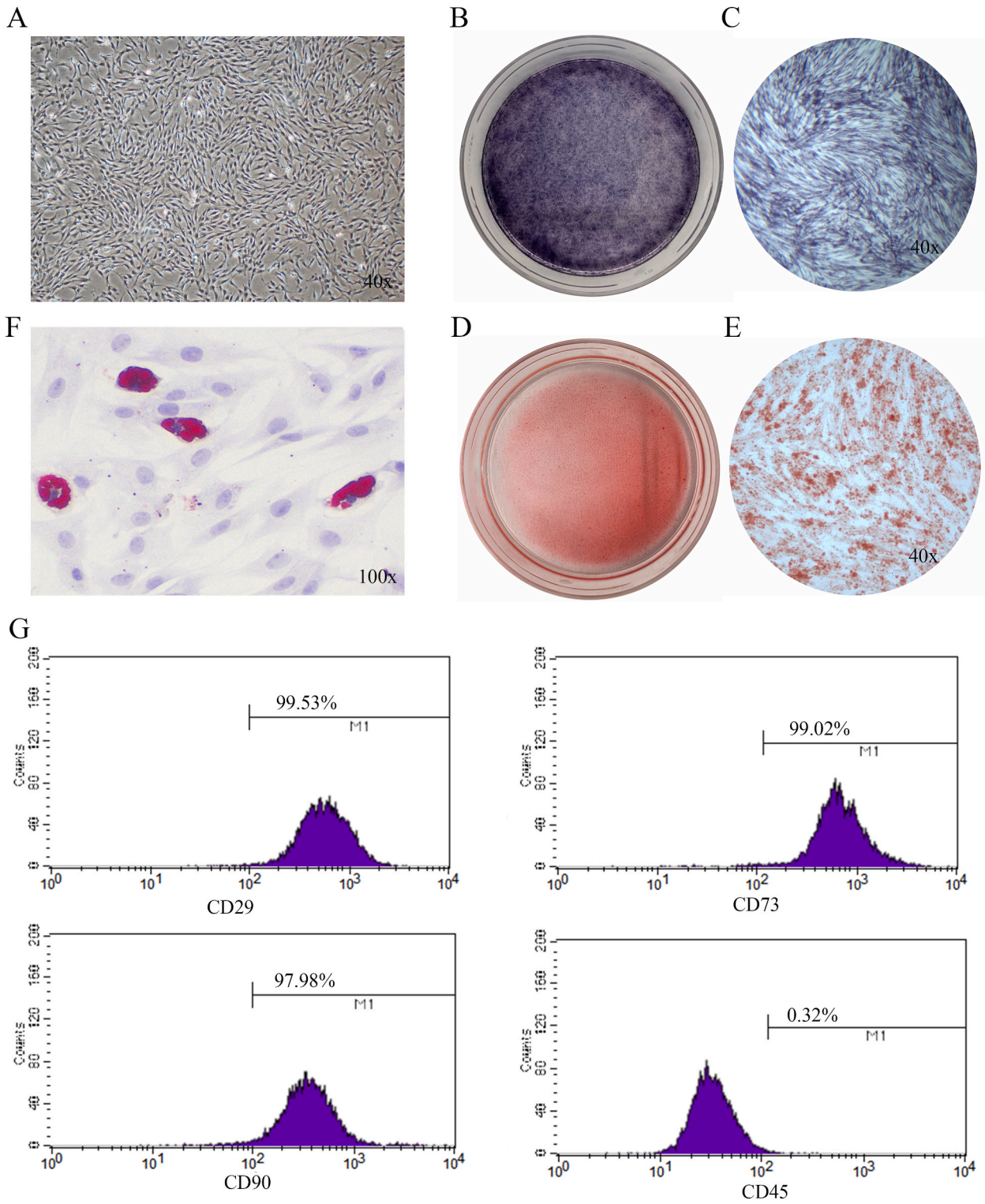

Fig. 1. Morphology and identification of hDPSC. (A) Morphology of primary hDPSC on 14 days. (B) ALP staining result after 2 weeks of osteogenic induction. (C) Image of ALP staining result under microscope. (D) Alizarin Red S staining result after 2 weeks of osteogenic induction. (E) Image of Alizarin Red S staining result under microscope. (F) Oil red O staining result after 2 weeks of adipogenic induction. (G) Flow cytometry results: hDPSC was positive for CD29, CD73, CD90 and negative for CD45. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.4. Psoriasis lesion area and severity index (PASI) score

As previous [26], the PASI score was a cumulative score (erythema score + scaling score + thickening score) used to measure the severity of back skin in mice. Erythema, scaling, and thickening were scored independently from 0 to 4: 0, none; 1, slight; 2, moderate; 3, marked; 4, very marked.

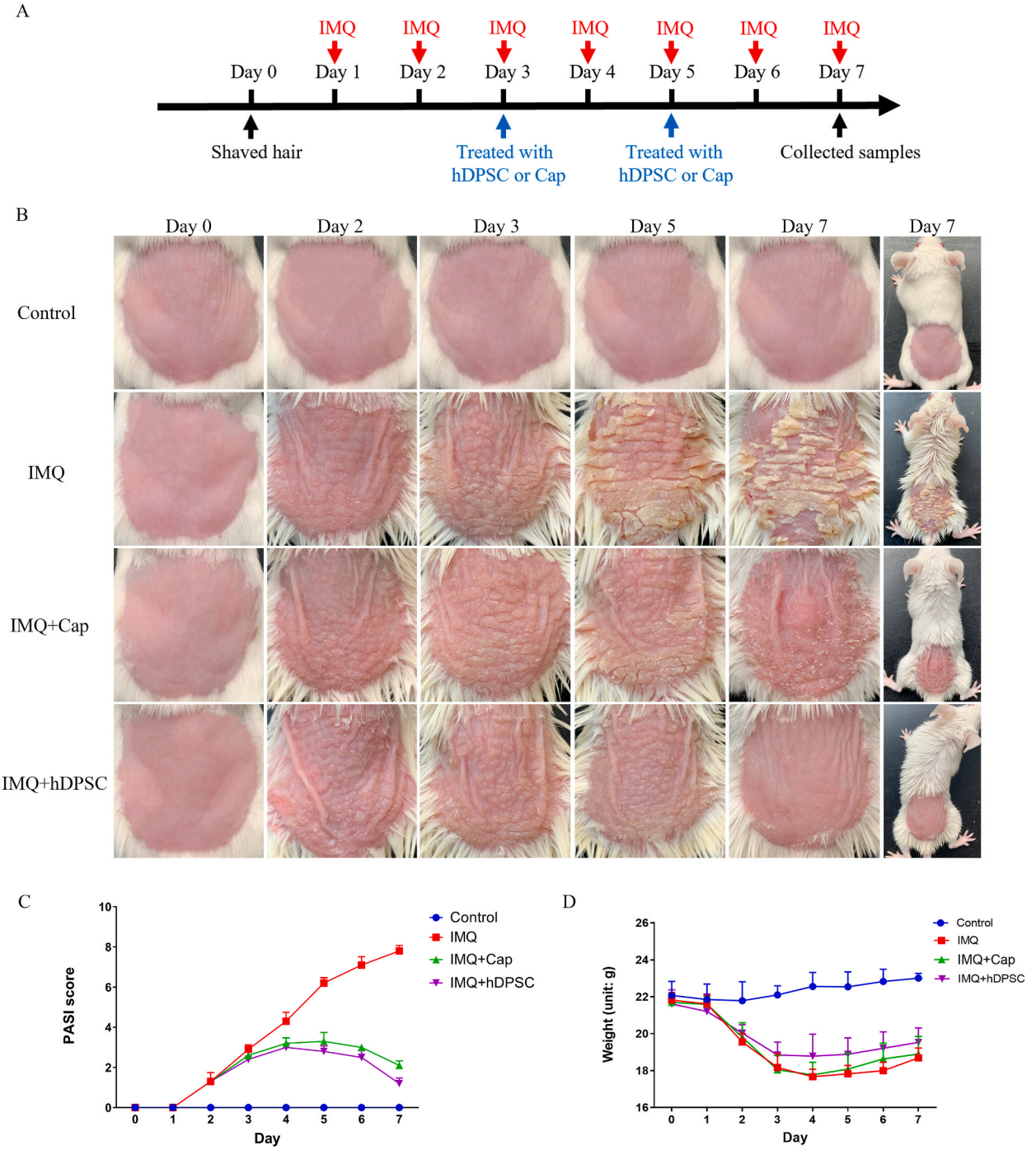

Fig. 2. Effect of hDPSC on skin lesions of IMQ-induced psoriasis in mice. (A) Experimental scheme and treatment of IMQ-induced psoriasis in mice. (B) Skin phenotype of 4 groups on day 0, 2, 3, 5, 7. (C) PASI score: erythema score + scaling score + thickening score. (D) The body weight of mice.

2.5. Histology, immunohistochemistry and fluorescence immunoassay

Hematoxylin-eosin (HE) staining, immunohistochemical (IHC) analysis and fluorescence immunoassay (FIA) were performed as previously described [11,27]. In brief, back skin samples in each group were fixed in 4% paraformaldehyde, embedded in paraffin and sliced (thickness, 5 μm), then stained with HE, incubated with antibody against keratin 16 (K16) for IHC and against CD4, CD11c for FIA. Four stained sections of each specimen were selected for observation and photography.

2.6. Enzyme-linked immunosorbent assay (ELISA)

Peripheral blood was collected before sacrifice on day 7. Referring to previous study [28], mice were anesthetized by inhalation of isoflurane (Lunan Better Pharmaceutical, China) in a plexiglass chamber to minimize pain and discomfort. Peripheral blood was collected by eyeball extraction method and placed in sterile EP tubes at room temperature for 30 min, then centrifuged at 4000 r/min for 15 min. The supernatant (blood serum) was taken for ELISA analysis. The concentrations of TNF-α, IFN-γ, IL-17 and IL-23 in serum were detected by the ELISA kit (TaKaRa, Japan) according to the manufacturer's instructions.

2.7. Quantitative real-time PCR (qPCR)

On day 7, the dorsal skin of mice was taken after sacrifice and the total RNA of dorsal skin was extracted by RNA Isolation Kit (Vazyme, China) according to the manufacturer's instructions. Reverse transcription was performed with PrimeScript RT Master Mix (TaKaRa, Japan) to synthesize first-strand cDNA. The mRNA expressions of TNF-α, IFN-γ, IL-17, IL-23, S100A8, S100A9 were measured by Viia 7 qPCR instrument (Applied Biosystems, USA) using SYBR Green Master Mix Reagent (Applied Biosystems, USA). The mRNA expression of GAPDH was used as an endogenous normalization control. Primer sequences were shown in Table 1.

2.8. Spleen size and weight

After mice were sacrificed under inhalation anesthesia on day 7, the spleen was taken, photographed and weighed to observe the changes of spleen in size and weight.

2.9. Statistical analysis

All experiments were performed at least three times. GraphPad Prism 7 software was used for statistical analysis. Firstly, determine whether the data had homogeneity and followed normal distribution. Shapiro-Wilk normality test was performed, and groups with $P > 0.05$ were accepted in the normal distribution. Groups in normal distribution were compared using Student's t-test (comparison between two groups) and One-way analysis of variance (comparison between multiple groups). The experimental data were expressed as mean ± SD, P values < 0.05 were considered statistically significant.

3. Results

3.1. Characteristics of the hDPSC

After 2 weeks of culture, primary hDPSC was isolated from pulp tissue by enzymatic digestion method. The morphology of hDPSC was similar to fibroblasts in spindle shape with strong proliferative capacity (Fig. 1A).

After 2 weeks of culture in osteogenic induction medium, ALP and Alizarin Red S staining results showed increased ALP activity and formation of mineralized nodules (Fig. 1B–E). In adipogenic induction medium, the presence of lipid droplets was observed by oil red O

Table 1
Primer sequences of this study.

Gene name		Primer sequences (5–3)
GAPDH	Forward	AGGTCGGTGTGAACGGATTTG
	Reverse	TGTAGACCATGTAGTTGAGGTCA
S100A8	Forward	TCCTTGCGATGGTGATAAA
	Reverse	GGCCAGAAGCTCTGCTACTC
S100A9	Forward	GACACCCTGACACCCTGAG
	Reverse	TGAGGGCTTCATTTCTCTTCTC
TNF-α	Forward	GAGAAGTTCCCAAATGGC
	Reverse	ACTTGGTGGTTTGCTACG
IFN-γ	Forward	TAACTCAAGTGGCATAGATGTGGAAG
	Reverse	GACGCTTATGTTGTTGCTGATGG
IL-17	Forward	TGCTACTGTTGATGTTGGGAC
	Reverse	AATGCCCTGGTTTTGGTTGAA
IL-23	Forward	GACTCAGCCAACTCCTCCAGCCAG
	Reverse	TTGGCACTAAGGGCTCAGTCAGA

staining (Fig. 1F). These results indicated that hDPSC could differentiate into osteocytes and adipocytes.

The third passage hDPSC was identified by flow cytometry. The results showed that cells were positive for hDPSC positive markers: CD29, CD73, CD90 and negative for leucocytes marker: CD45 (Fig. 1G).

3.2. hDPSC ameliorated the symptoms of skin lesions in IMQ-induced psoriasis

After 7 days of consecutive application of Vaseline on the dorsal skin in Control group, these mice had smooth skin without erythema, scaling and thickening. In IMQ group, mice started to display erythema, scaling and thickening on day 2 of consecutive application of IMQ cream, and reached the most severe skin lesions on the day 7. And the mice showed depression, decreased appetite, reduced activity, increased oil secretion throughout the body, and emaciation (Fig. 2B).

In order to explore whether hDPSC could ameliorate IMQ-induced psoriasis in mice, hDPSC was injected subcutaneously on the back of mice on day 3 and day 5 after IMQ cream application. The positive control group was topically treated with Cap on day 3 and day 5 after IMQ cream application. The photographs were taken and showed that both IMQ + Cap and IMQ + hDPSC groups could improve the psoriasis skin phenotype compared with the IMQ group. IMQ + hDPSC group was closer to normal skin than IMQ + Cap (Fig. 2B).

The PASI score based on the symptoms of erythema, scaling, and thickening showed that hDPSC and Cap had a significant preventive effect on the development and severity of psoriasis (Fig. 2C). The body weight of mice was measured from day 0 to day 7 (a total of 8 days). IMQ-induced psoriasis could lead to significant body weight loss in mice. The weight loss in IMQ + hDPSC group was lower than that in IMQ and IMQ + Cap groups (Fig. 2D).

3.3. hDPSC decreased the abnormal epidermal proliferation in IMQ-induced psoriasis

All mice were sacrificed on day 7. The skin of dorsal lesions was taken for histopathological study. HE staining results showed that the epidermis was thin in the Control group (Fig. 3A). IMQ group showed the characteristic skin changes associated with psoriasis, such

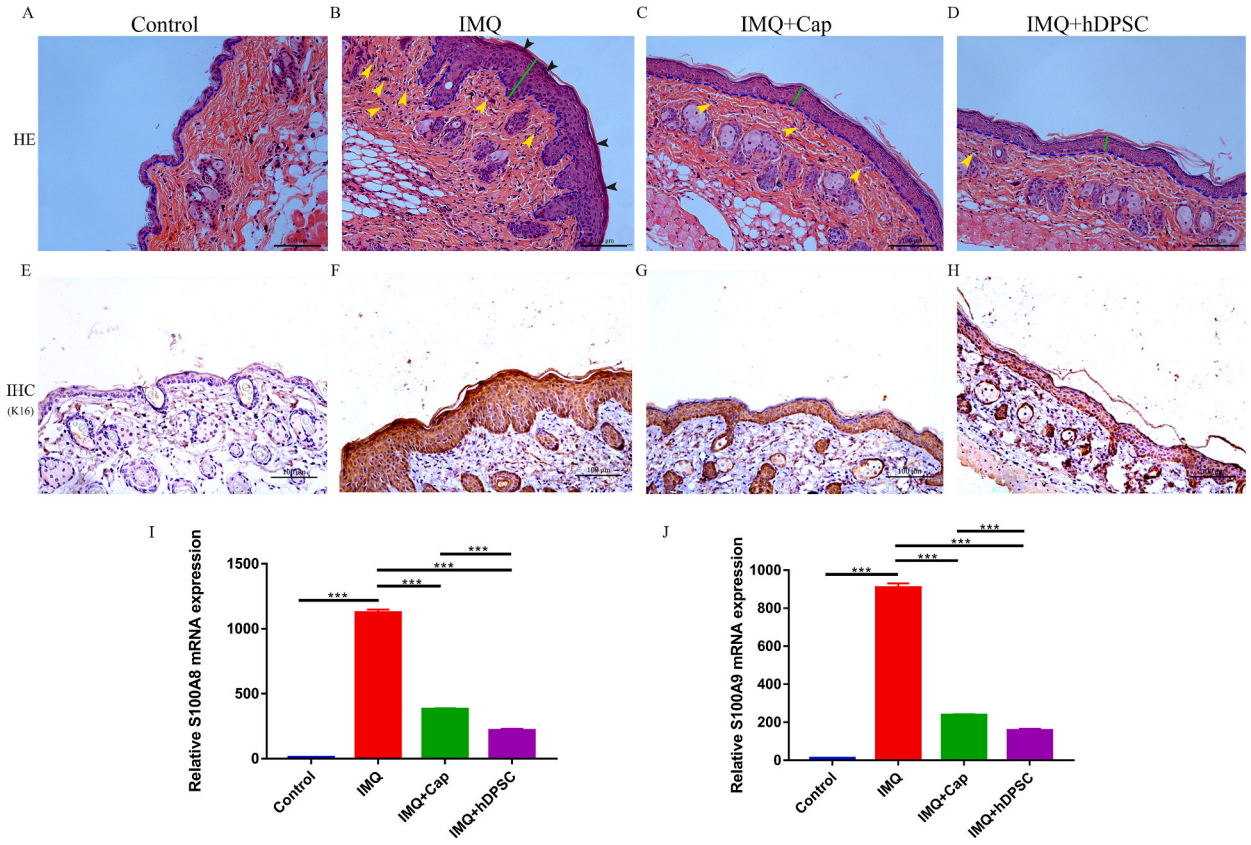

Fig. 3. Effect of hDPSC on abnormal epidermal proliferation of IMQ-induced psoriasis in mice. **(**A–D) HE staining revealed the epidermal hyperplasia (green bar), parakeratosis (black arrows), and inflammatory infiltrates (yellow arrows) in skin lesions on day 7. (E–H) IHC analysis of the K16 expression in skin lesions on day 7 (DAB staining). (I) The mRNA expression of S100A8 in skin lesions. (J) The mRNA expression of S100A9 in skin lesions. *P < 0.05; **P < 0.01; ***P < 0.001. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

as significant thickening of the epidermis, excessive extension of the epidermis into the dermis, parakeratosis, inflammatory cells infiltration (Fig. 3B). After treatment with Cap and hDPSC, epidermal thickness, parakeratosis and inflammatory cells infiltration were significantly reduced compared with IMQ group (Fig. 3C and D). IHC staining results showed that the expression of K16 related to epidermal proliferation [29] was significantly increased in IMQ group compared with Control group (Fig. 3E and F), While decreased in IMQ + Cap and IMQ + hDPSC groups (Fig. 3G and H). The expressions of S100A8 and S100A9 associated with epithelial proliferation [30] were detected by qPCR. The results showed that the S100A8 and S100A9 expressions were significantly increased in IMQ group compared with the Control group ($P < 0.001$), while significantly decreased in the IMQ + Cap and IMQ + hDPSC groups ($P < 0.001$). And IMQ + hDPSC group had the most significant decrease compared with IMQ + Cap group ($P < 0.001$). Results were shown in Table 2 and Fig. 3I and J.

3.4. hDPSC suppressed the local and systemic immune responses in IMQ-induced psoriasis

After the mice were sacrificed on day 7, the skin of dorsal lesions was taken to observe the changes of local immune response by FIA and qPCR. The FIA results showed that the ratio of subepithelial $CD4^+$ T-lymphocytes (Th cells) and $CD11c^+$ dendritic cells were significantly enhanced in the IMQ group (Fig. 4A, B, E, F), while reduced in the IMQ + hDPSC and IMQ + Cap groups (Fig. 4C, D, G, H). Th cells (especially Th1 and Th17 cells) and dendritic cells are important immune cells involved in the pathogenesis of psoriasis [31]. qPCR was used to detect the expression of subepithelial inflammatory factors, and the results showed that the mRNA expressions of Th1 cytokines (TNF-α, IFN-γ) and Th17 cytokines (IL-17, IL-23) were increased in IMQ group ($P < 0.01$), while Cap or hDPSC treatment effectively decreased these mRNA expression levels ($P < 0.05$). Results were shown in Table 3 and Fig. 4I–L.

Spleen and serum were taken to observe the changes of systemic immune response. The spleen, an important immune organ in mice, was significantly enlarged in size and weight in the IMQ and IMQ + Cap group, while hDPSC suppressed the splenomegaly ($P < 0.01$, Fig. 4M and N). This suggested that although Cap could alleviate the skin lesions in IMQ-induced psoriasis to some extent, it didn't suppress the splenomegaly. The inflammatory factors associated with psoriasis in peripheral blood were detected by ELISA. Results showed that the concentrations of TNF-α and IFN-γ in serum in the IMQ + hDPSC group were significantly lower than those in the IMQ and IMQ + Cap groups ($P < 0.05$). There was no significant difference in IL-17 and IL-23 concentrations among the four groups ($P > 0.05$). Results were shown in Table 3 and Fig. 4O–R.

4. Discussion

Psoriasis is a chronic autoimmune-mediated skin disease associated with local and systemic inflammation responses and has a significant impact on quality of life, with some patients even thinking of suicide [3,32]. Although the pathogenesis of psoriasis is not well understood, it has been shown that psoriasis is related to genetic and epigenetic factors, in which the immune system plays an important role [33]. hDPSC has unique immunoregulatory potentials in vitro and in vivo, and hDPSC exerts immunosuppression effects mainly through interacting with the innate and acquired immune systems. hDPSC inhibits the maturation of DC cells (primary antigen-presenting cells), decreases the antigen presentation ability of DC cells, and ultimately leads to T cell dysfunction [15]. hDPSC impairs proliferation of NK cells and promotes their apoptosis, thereby inhibiting the release of large amounts of inflammatory factors by NK cells (such as IFN-γ, TNF-α) and toxic effects, ultimately suppressing the body's immune response [18]. hDPSC is able to inhibit the proliferation of T and B lymphocytes [34]. In addition, hDPSC can release soluble immunosuppressive molecules such as PGE2, TGF-β and NO in a paracrine manner to suppress the T cell inflammatory response [26]. hDPSC lacks major histocompatibility complex (MHC), which makes it safe to use in an allogeneic environment without the risk of immune rejection [35]. Thus, hDPSC therapy may be an alternative option for autoimmune diseases. In our study, hDPSC was injected subcutaneously on the back lesions of mice, and we found that hDPSC could reduce the symptoms of skin lesions and suppress local and systemic immune responses of IMQ-induced psoriasis in mice.

The therapeutic effect was similar to that of Kim CH et al. (2019) [25], the difference was that they used embryonic stem cells, while hDPSC was used in this study, which are easier to obtain, and have less ethical concerns.

Several studies have also reported the positive effects of other types of mesenchymal stem cells (MSC) in psoriasis, such as human embryonic stem cells-derived MSC (hE-MSC) [25] and human umbilical cord derived MSC (hUC-MSC) [5,36]. However, ethical controversies have been surrounding hE-MSC. To obtain hE-MSC, the 5-day-old preimplantation embryo must first be destroyed. Minimizing the risk of harm, obtaining informed consent, reducing the potential for therapeutic misconception, and facilitating sound translation from experimental to the clinical stage are all challenges for hE-MSC [37,38]. As for hUC-MSC, the main challenge is immunogenic concerns when used heterogeneously. For autologous applications, the umbilical cord must be properly cryopreserved for an extended period of time after delivery [39]. Another significant problem is that there are not enough cells in adults [40]. Bone marrow mesenchymal stem cell (BMSC) and adipose-derived stem cell (ADSC) are potential candidates for psoriasis therapy [9,41]. BMSC has been the most thoroughly studied and is considered the gold standard for clinical applications. However, the isolation

Table 2
The mean and SD values of S100A8 and S100A9 mRNA relative expressions obtained from qPCR.

	Control	IMQ	IMQ + Cap	IMQ + hDPSC
S100A8	1.0 ± 0.0	1124.1 ± 24.7	380.8 ± 7.4	218.6 ± 10.8
S100A9	1.0 ± 0.0	908.4 ± 21.5	237.9 ± 4.2	157.3 ± 9.2

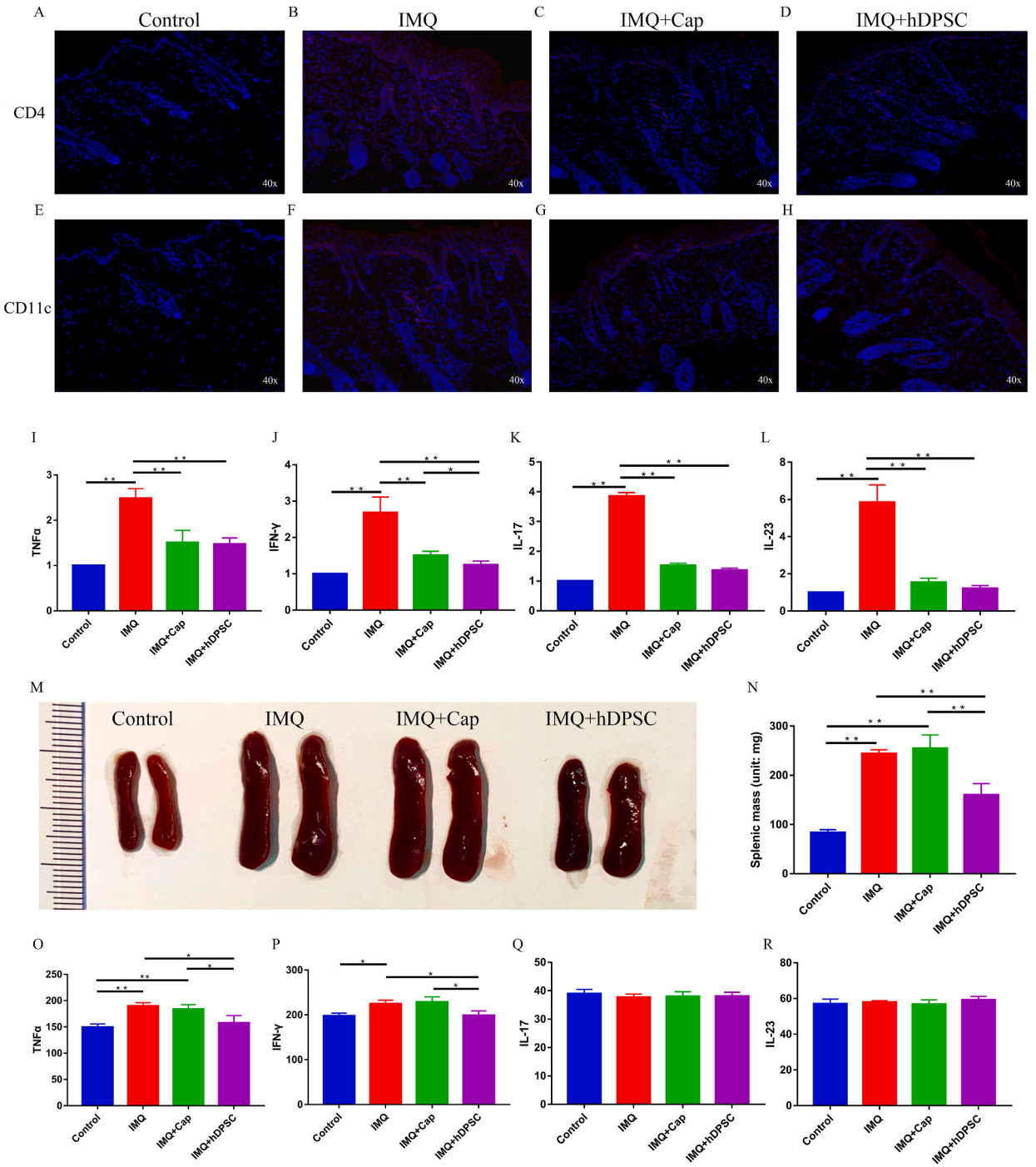

Fig. 4. Effect of hDPSC on local and systemic immune responses of IMQ-induced psoriasis in mice. (A–D) FIA analysis of the $CD4^+$ T-lymphocytes ratio (red fluorescence). (*E*–H) FIA analysis of the $CD11c^+$ dendritic cells ratio (red fluorescence). (I–L) The mRNA expressions of subepithelial inflammatory factors (TNF-α, IFN-γ, IL-17, and IL-23). (M) The size of spleen. (N) The weight of spleen. (O–R) The mRNA expressions of inflammatory factors in serum (TNF-α, IFN-γ, IL-17, and IL-23). *P < 0.05; **P < 0.01; ***P < 0.001. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

process of BMSC is painful and invasive, the proportion of MSC in bone marrow is low, and their potency may be lost in the elderly or patients with certain diseases and disorders [39,42]. Moreover, the proliferation and differentiation ability of BMSC decreased with age [43]. ADSC has several advantages over BMSC. ADSC can be easily obtained from the subcutaneous area by minimally invasive and painless methods with high yield [44]. ADSC has strong proliferation ability and can maintain its phenotype for a longtime during

Table 3
The mean and SD values of TNF-α, IFN-γ, IL-17 and IL-23 expression levels in skin (qPCR) and serum (ELISA).

	Control	IMQ	IMQ + Cap	IMQ + hDPSC
Skin (qPCR)				
TNF-α	1.0 ± 0.0	2.5 ± 0.2	1.5 ± 0.2	1.5 ± 0.1
IFN-γ	1.0 ± 0.0	2.7 ± 0.4	1.5 ± 0.1	1.2 ± 0.1
IL-17	1.0 ± 0.0	3.9 ± 0.1	1.5 ± 0.1	1.3 ± 0.1
IL-23	1.0 ± 0.0	5.9 ± 0.9	1.5 ± 0.2	1.2 ± 0.2
Serum (ELISA)				
TNF-α	149.7 ± 6.1	190.0 ± 6.2	183.7 ± 8.8	157.6 ± 8.3
IFN-γ	197.9 ± 5.9	224.6 ± 8.1	228.8 ± 9.7	199.1 ± 8.6
IL-17	38.9 ± 1.5	37.7 ± 1.1	38.0 ± 1.6	38.1 ± 1.3
IL-23	56.9 ± 2.7	57.9 ± 0.9	56.8 ± 2.4	59.2 ± 1.9

culture, which may be more suitable for allotransplantation than BMSC [45]. However, S. Wu et al. (2019) [46] found the carcinogenic role of ADSC. ADSC may stimulate the growth, migration and invasion of tumors by secreting transforming growth factor (TGF-β) and stem cell factor (SCF). Compared with other types of MSC, hDPSC is abundant in source and can be obtained from impacted third molars, orthodontically extracted teeth. In particular, the deciduous teeth lost during physiological processes are less ethically controversial, more readily available, and a painless and non-invasive procedure for the donors [47]. Due to the origin of the neural crest, hDPSC possesses unique neurogenic potential that is not found in other types of MSC [48]. hDPSC has a higher clonal formation ability than BMSC and ADSC, and the proliferation rate of hDPSC is 30–50 times that of BMSC [49]. It is easy to obtain a large number of cells in a short time to meet the clinical needs. Compared with ADSC, hDPSC shows higher expression of angiogenesis related genes and secretion of vascular endothelial growth factor [50]. However, the viability, proliferation and differentiation potential of hDPSC decreased with age [51].

Human dental pulp stem cells derived from exfoliated deciduous teeth (SHED) have advantages over those from permanent teeth because exfoliated deciduous teeth are easy to obtain, fall off naturally, do not involve invasive methods, and it guarantees patient safety with limited legal and ethical considerations [47,52,53]. However, in our study, hDPSC of permanent teeth was chosen over SHED because healthy SHED is often not accessible due to the high incidence of caries and pulpitis in deciduous teeth [53]. And, due to the natural absorption of the root of deciduous teeth, the pulp of the lost teeth is easily exposed to the mouth and contaminated by bacteria in the mouth, leading to the failure of SHED culture. Moreover, there is less dental pulp tissue in the pulp cavity of deciduous teeth compared with permanent teeth. In contrast, hDPSC in permanent teeth usually derives from the teeth that have been removed for treatment, such as impacted third molars that have no chewing function in the mouth and may lead to recurrent pericoronitis, caries in adjacent teeth. Most young adults have impacted teeth and need to be removed. Impacted teeth in young adults have more pulp tissue in the pulp cavity. And hDPSC from young adults has stronger stem cell properties.

IL-23/Th17/IL-17 axis and Th1/IFN-γ axis play a key role in psoriasis inflammation [8,9,54]. Therefore, TNF-α, IFN-γ, IL-17 and IL-23 expressions were increased in IMQ group in the skin and serum. The immunomodulatory capacity of hDPSC is achieved by downregulating Th1 and Th17 [55,56]. Thus, it was reasonable that Th1 and Th17 cytokine levels (TNF-α, IFN-γ, IL-17 and IL-23) were decreased in IMQ + hDPSC group in skin. However, there was no significant difference in IL-17 and IL-23 among the four groups on day 7 in serum (Fig. 4Q and R). van der Fits L et al. (2009) reported that the expressions of IL-17 and IL-23 were transient, reaching the maximum expression on day 3 after IMQ induction and subsequently decreasing [26].

In this study, we also found that although Cap could alleviate epidermal proliferation and reduce the symptoms of psoriasis skin lesions in IMQ-induced mice, it could not decrease the TNF-α and IFN-γ expressions in serum and suppress the splenomegaly. Liang W et al. (2017) reported that Cap inhibited cell proliferation and normalized keratinocyte differentiation by downregulating STAT1 and STAT3 signaling pathways, thereby alleviating the symptoms of psoriasis skin lesions [57]. We speculate that Cap ameliorates psoriasis skin lesions by inhibiting epidermal proliferation, possibly independent of immune and inflammatory responses. And the therapeutic effect of hDPSC seems to be superior to Cap to some extent, which provides experimental basis for clinical treatment of psoriasis.

Due to the ethical consideration and the limited availability of human materials, animal models of psoriasis are of great importance for studying the pathogenesis and therapeutic principles of psoriasis. The best way would be to identify a naturally occurring animal disease similar to human psoriasis. However, apart from humans, psoriasis-like disease occurs only in Rhesus Monkeys [58] and English Springer Spaniels [59]. These two cases are sporadic and appear to be rare and therefore not suitable for systematic studies [60]. Applying key molecules to healthy organisms would be another good way to generate animal models of psoriasis. Among them, IMQ is an agonist of Toll-like receptors 7 and 8 (TLR7, TLR8), and daily topical application can cause psoriasis-like disease [61]. Besides, mice are cheap and easy to obtain. Therefore, the model of IMQ-induced psoriasis in mice has been widely used in new therapies. However, IMQ-induced mice model has only been used to study the early stages of psoriasis. As reported [62,63], the most severe skin lesions were reached on day 7 after consecutive application of IMQ cream. On day 8 or day 9, when IMQ cream was applied to mice, symptoms of dorsal lesions improved and skin inflammation decreased in IMQ group. We speculate that IMQ induces transient inflammatory outburst, and the model may develop adaptation and tolerance to drugs under the consecutive application of IMQ. Therefore, this study cannot evaluate longtime treatment effect. In addition, this study used a drug-induced psoriasis animal model, which differs from human psoriasis in etiology and course. And this model is an acute model that does not adapt to long-term treatment and does not fully reproduce the long-term inflammatory features of psoriasis in patients [64]. Therefore, the effectiveness of hDPSC for the treatment of psoriasis and related mechanisms still need further investigated.

5. Conclusions

In summary, our study demonstrated that hDPSC could reduce the symptoms of skin lesions and suppress local and systemic immune responses of IMQ-induced psoriasis in mice. The therapeutic effect of hDPSC seemed to be superior to Cap to some extent.

Author contribution statement

Wen Kang: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Li Wu: Performed the experiments; Wrote the paper.
Cheng Chen; Weige Xie; Jiaqi Chen; Lingyan Huang: Performed the experiments.
Shiyu Song; Hongwei Wang: Contributed reagents, materials, analysis tools or data.
Sijing Xie: Conceived and designed the experiments.

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Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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