



# Effects of different concentrations of human umbilical cord mesenchymal stem cells to ameliorate psoriasis-like skin lesions in BALB/c mice

Youshu Lin<sup>1#</sup>, Hongwei Wang<sup>2#</sup>, Cuibao Jiang<sup>2</sup>, Chen Chen<sup>2</sup>, Danpeng Shen<sup>2</sup>, Fan Xie<sup>2</sup>, Hongpeng Zhang<sup>2</sup>, Jie Yang<sup>1^</sup>, Hao Wang<sup>2</sup>

<sup>1</sup>Department of Cosmetic Dermatology, The Fifth People's Hospital of Hainan Province, Haikou, China; <sup>2</sup>Asia Stem Cell Regenerative Pharmaceutical Co., Ltd., Shanghai, China

**Contributions:** (I) Conception and design: J Yang; (II) Administrative support: Hao Wang; (III) Provision of study materials or patients: Hongwei Wang, C Jiang, H Zhang; (IV) Collection and assembly of data: Y Lin; (V) Data analysis and interpretation: C Chen, D Shen, F Xie; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

<sup>#</sup>These authors contributed equally to this work and should be considered as co-first authors.

**Correspondence to:** Jie Yang. Department of Cosmetic Dermatology, The Fifth People's Hospital of Hainan Province, Haikou, China.

Email: 13337564169@163.com; Hao Wang. Asia Stem Cell Regenerative Pharmaceutical Co., Ltd., Shanghai, China.

Email: wanghao@xibaozhiliao.cn.

**Background:** Psoriasis is an immune-mediated chronic, recurrent, inflammatory skin disease. In view of the research on the relationship between stem cells and the pathogenesis of psoriasis, stem cells may be a new breakthrough in the systemic treatment of psoriasis.

**Methods:** The BALB/c mouse psoriasis-like model induced by imiquimod was established and animals were randomly divided into a control group, model group, human umbilical cord mesenchymal stem cells (hUC-MSCs) group with different concentrations (injected separately with umbilical cord stem cells  $1 \times 10^7$ /kg,  $2 \times 10^7$ /kg,  $4 \times 10^7$ /kg through the caudal vein) and fresh hUC-MSCs group (injected with fresh umbilical cord stem cells  $2 \times 10^7$ /kg through the tail vein). The Psoriasis Area and Severity Index (PASI) score was used to observe the changes in skin lesions. The epidermal thickness, degree of keratinization and infiltration of inflammatory cells were observed by HE staining. The concentrations of *TNF- $\alpha$* , *IFN- $\gamma$* , *IL-17A*, *IL-23* and other cytokines in serum and skin of mice were measured by enzyme-linked immunosorbent assay (ELISA).

**Results:** Mice treated with hUC-MSCs showed a good dose-response dependence compared with the control group. As the concentration of hUC-MSCs increased, so did the spleen index. According to the PASI integral trend chart, hUC-MSCs can delay the appearance of skin lesions and accelerate the recovery of skin lesions. HE staining showed that the number of parakeratosis cells in the hUC-MSCs treatment group was significantly decreased, and the degree of dermal hyperplasia and inflammatory cell infiltration in erythrocyte extravasation was significantly lower than in the model group. The higher the concentration of hUC-MSCs, the lower the concentration of the four cytokines in serum and skin tissue.

**Conclusions:** hUC-MSCs had an obvious therapeutic effect on imiquimod-induced psoriasis in mice, and a high concentration of hUC-MSCs had the best therapeutic effect. This effect intensity is dose-dependent, and hUC-MSCs at high concentrations have better therapeutic effect.

**Keywords:** Cytokines; indirubin; human umbilical cord mesenchymal stem cells (hUC-MSCs); psoriasis

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<sup>^</sup> ORCID: 0000-0002-2583-0225.

## Introduction

Psoriasis is an immune-mediated chronic, recurrent, inflammatory skin disease with typical clinical manifestations of scaly erythema or plaques that can be localized or widely distributed (1). The etiology of psoriasis involves genetic, immune, environmental and other factors (2,3). Psoriasis leads to excessive keratinocyte proliferation or inflammation of synovial cells and chondrocytes through an immune response mainly mediated by T lymphocytes and jointly participated in by multiple immune cells (4,5).

The treatment plan for psoriasis is determined according to the patient's symptoms, with external treatment as the mainstay for mild psoriasis, systematic treatment for moderate and severe psoriasis, and targeted biologics as the appropriate treatment for patients with poor response to traditional systemic drug therapy (5,6). The clinical PASI scoring system was used to determine the degree of psoriasis. Since the area of skin lesions was artificially interfered, the score was mainly based on the severity of skin lesions. The erythema, scaling, and infiltration were scored separately as 0 (not present); 1 (slight); 2 (moderate); 3 (severe); and 4 (extremely severe), from which, the scores were summed up for a total score.

Systemic therapeutic drugs currently used mainly include methotrexate, cyclosporine, retinoids, azathioprine, mofetilumab and other immunosuppressants (7-11), but the use of immunosuppressants is often limited by adverse drug reactions, so long-term drug administration is not possible (12). In recent years, a series of biologic agents and small molecule drugs with specific targets for psoriasis have been developed one after another, and they have achieved good clinical efficacy, but the long-term efficacy and safety remain to be observed, and common characteristics such as efficacy attenuation may lead to treatment failure. Therefore, the treatment of psoriasis still needs more options.

Psoriasis is associated with abnormal keratinocytes, immune cells, hematopoietic stem cells (HSCs), vascular endothelial cells, and bone marrow mesenchymal stem cells (MSCs) (13,14). HSCs from patients with psoriasis produce subsets of T cells with a tendency to secrete *Tb1* cytokines and subsets of dysfunctional Treg cells. Once activated by internal or external environmental factors, psoriatic T cells are prone to a *Tb1* immune response, which not only induces epidermal stem cells to produce more transient amplifying cells, eventually leading to overproliferation of keratinocytes, but can also promote the transformation

of MSCs into a pro-inflammatory cell MSC1 phenotype, contribute to angiogenesis and infiltration of psoriatic skin lymphocytes by inflammatory cytokines and chemokines, and aggravate the local *Tb1* immune response (15-17). MSCs have broad clinical application prospects, and can be used to treat diseases of nervous system, liver and kidney injury, autoimmune diseases, ischemic vascular diseases, diabetes complications and tumors. In addition, mesenchymal stem cells have been found in umbilical cord blood, periodontal ligaments, dermis, skeletal muscle, placenta and pancreas.

In view of the research on the relationship between stem cells and the pathogenesis of psoriasis, stem cells may be a new breakthrough in the systemic treatment of psoriasis. However, it is necessary to investigate the effective concentration of stem cells in the treatment of psoriasis while observing their therapeutic effect. We investigated the efficacy and effective concentration of human MSCs in delaying psoriatic lesions in mice *in vitro*. Because changes in the levels of cytokine can indirectly reflect the changing trend of skin lesions, four cytokines, interferon (*IFN*)- $\gamma$ , tumor-necrosis factor (*TNF*)- $\alpha$ , interleukin (*IL*)-17 and *IL*-12, were selected to reflect the effect of the stem cell treatment. We found that hUC-MSCs (human umbilical cord mesenchymal stem cells) have a therapeutic effect on imiquimod-induced psoriasis in mice by inhibiting the concentration of *IL*-23 and *IL*-17 factors, and may become a new therapeutic approach for clinical treatment of psoriasis.

We present the following article in accordance with the ARRIVE reporting checklist (available at <https://atm.amegroups.com/article/view/10.21037/atm-22-4/rc>).

## Methods

### Main reagents and materials

Imiquimod 5% cream (batch no. 17010139), purchased from Sichuan Mingxin Lidi Company; Recombinant Mouse Interleukin 12 (*rmIL*-12 *p70*), BD Pharmingen Inc., batch no. 6278771, Specifications: 5  $\mu$ g; lipopolysaccharide (LPS) lyophilized powder (Sigma, Inc., batch no. 045M4087V, specification: 10 mg); enzyme-linked immunosorbent assay (ELISA) kits for IL-17A, IL-12 (Shanghai Future Industry Co., Ltd., batch no. JL20171, JL20166). Compound electrolyte solution (Shanghai Kelun Pharmaceutical Co., Ltd., batch no. T14102120). Human umbilical cord MSCs (hUC-MSCs) were produced and prepared by the GMP laboratory of Shanghai Quansheng Biotechnology Co., Ltd.

The UC donor gave signed informed consent to donate for scientific research and passed the infectious disease test. The collected UC tissues were cut and separated by slippage. After generation and amplification, they were prepared into  $1 \times 10^8$  cells/mL normal saline cell suspension (batch no. 20191201). After passing the quality inspection of sterility, mycoplasma, endotoxin and flow phenotype, they were used in this experiment. The experimental instruments were provided by Shanghai Quan Sheng Biotechnology Co., Ltd.

### *Experimental animals*

#### **Statement of ethics**

Experiments were performed under a project license (No. 2016LL003) granted by institutional board of The Fifth People's Hospital of Hainan Province, in compliance with The Fifth People's Hospital of Hainan Province guidelines for the care and use of animals.

We purchased 60 SPF grade 6–8 weeks-old male BALB/c mice weighing about 20 g from Beijing Vitong Lihua Experimental Animal Co., Ltd. The animals were kept in the laboratory for at least 1 week and then tested under standard rearing conditions (room temperature 25–27 °C) with alternating light and dark cycles of 12 h. The mice were allowed free access to food (standard pellet feed) and water.

### *Experiment design*

Psoriasis-like lesions were induced in the BALB/c mice by applying 5% imiquimod cream at 62.5 mg/day on the back of the mice for consecutive 7 days, as well as subcutaneously injecting 10 ng (5  $\mu$ L) of recombinant mouse interleukin (*rmIL-12*) and 20  $\mu$ g (5  $\mu$ L) of LPS once at the beginning of model creation. During drug intervention, imiquimod 5% cream was applied every other day to avoid spontaneous regression of the skin lesions.

The BALB/c mice were randomly divided into six groups of 10 mice. Group A: normal control group; Group B: model group; Group C1: injected with UC stem cells ( $1 \times 10^7$ /kg) through the caudal vein; C2 group: injected with UC stem cells  $2 \times 10^7$ /kg through the caudal vein; C3 group: injected with UC stem cells  $4 \times 10^7$ /kg through the caudal vein; Group D: injected with fresh UC stem cells  $2 \times 10^7$ /kg through the tail vein. All mice were humanely killed after observation on the 7th day, and blood and dorsal skin tissue were collected for later use. The spleen was excised and weighed, and the spleen index (spleen mass/body mass) was calculated. Photographs were taken and recorded.

### *Cell stemness identification*

#### **Flow cytometry**

The second-generation cells were collected and digested with 0.25% TrypLE trypsin (Gibco), and the appropriate amount of cells was incubated with CD73, CD90, CD105, CD34, CD45 and HLA-DR antibodies (1  $\mu$ g/100  $\mu$ L, CST) that were coupled with PE (phycoerythrin) luminescent group on ice for 30 min. Cell surface antibody levels were detected by flow cytometry (cytoFLEX, Beckman-Coulter).

#### **Osteogenesis**

hUC-MSCs were seeded into each well of a 6-well plates at a density of  $1 \times 10^5$  cells/well. Next, 10 mmol/L  $\beta$ -glycerophosphate, 1  $\mu$ mol/L dexamethasone and 50  $\mu$ mol/L vitamin C were added to conventional medium for osteogenesis induction. The cells were cultured in the osteogenic induction medium for 4 weeks, and mineral bone nodules were detected with alizarin red S dye.

#### **Adipogenesis induction**

hUC-MSCs cells were seeded into each well of a 6-well plates at a density of  $1 \times 10^5$  cells/well. Next, 0.5 mmol/L 3-isobutyl-1-methylxanthine, 200  $\mu$ mol/L indomethacin, 1  $\mu$ mol/L dexamethasone and 10  $\mu$ mol/L insulin were added to conventional medium for adipogenesis induction. After cells were cultured in the adipogenic induction medium for 1 week, lipid droplets were detected with oil red O dye.

#### **Chondroblast induction**

hUC-MSCs cells were seeded into each well of a 6-well plates at a density of  $1 \times 10^5$  cells/well. Conventional medium was supplemented with 10  $\mu$ g/L transforming growth factor- $\beta$ 1, 0.1  $\mu$ mol/L dexamethasone, 100  $\mu$ mol/L ascorbic acid, 200 mmol/L glutamine and 1% Insulin-Transferrin-Selenium (ITS) as the medium for chondrogenic induction.

### *Immunohistochemical assay*

The dorsal skin tissue was fixed with 4% paraformaldehyde, embedded with paraffin and sectioned at 5  $\mu$ m thickness. The tissue sections were placed on slides and baked in a thermostat at 60 °C for 1 h until the wax on the surface of the tissue melted. The slides were dewaxed in xylene for 15 min, then soaked in 100% ethanol for 5 min, then in 95%, 90%, 80% and 70% ethanol for 2 min respectively, and washed with phosphate-buffered saline (PBS) three times for 3 min each time. The tissue sections were placed

**Table 1** Psoriasis area and severity index (PASI)

Items	Details
Erythema	0 point: none
	1 point: slight
	2 points: moderate
	3 points: severe
Induration/thickness	4 points: very severe
	0 point: none
	1 point: slight
	2 points: moderate
Desquamation/scaling	3 points: severe
	4 points: very severe
	0 point: none
	1 point: slight
	2 points: moderate
	3 points: severe
	4 points: very severe

in a citrate buffer and boiled in a pressure cooker for 3 min for antigenic repair. They were washed with distilled water and PBS twice successively, 3 min each time. The section was sealed with 5% bovine serum protein for 10 min, then the specific primary antibody was added to the section before incubation overnight at 4 °C. The section was washed three times with PBS for 5 min each time, before secondary antibody was added and incubation at 37% for 1 h, and then washed with PBS three times for 5 min each time. DAB was added, color developed for 5 minutes, and then the slides were rinsed with distilled water, before being dyed with hematoxylin for 10 s. The sections were dehydrated by gradient ethanol for 2 min each and made transparent by xylene for 5 min. Finally, the sections were sealed with resin for observation and photographing under a microscope.

#### Appearance score test

Psoriasis Area and Severity Index (PASI) were used to evaluate the appearance of the psoriasis lesions (Table 1). Since the area of skin lesions was artificially interfered, the score was mainly based on the severity of skin lesions.

#### Pathological evaluation

The dorsal skin tissue of the mice was fixed with 4% paraformaldehyde, embedded with paraffin and sectioned at 5 μm thickness. All tissue sections were stained with hematoxylin-eosin (HE). The skin thickness was measured after photographing.

#### Immunological evaluation

Serum and the dorsal skin of the mice were collected and stored at -80 °C. The tissues were homogenized at 4 °C, and the supernatant was collected and stored at -20 °C. The concentrations of *Tb1* and *Tb17*-related immune molecules tumor-necrosis factor (*TNF*)-α, interferon (*IFN*)-γ, *IL-17A* and *IL-23* in the serum and dorsal skin tissues were measured by ELISA kits. Procedures followed the manufacturer's instructions.

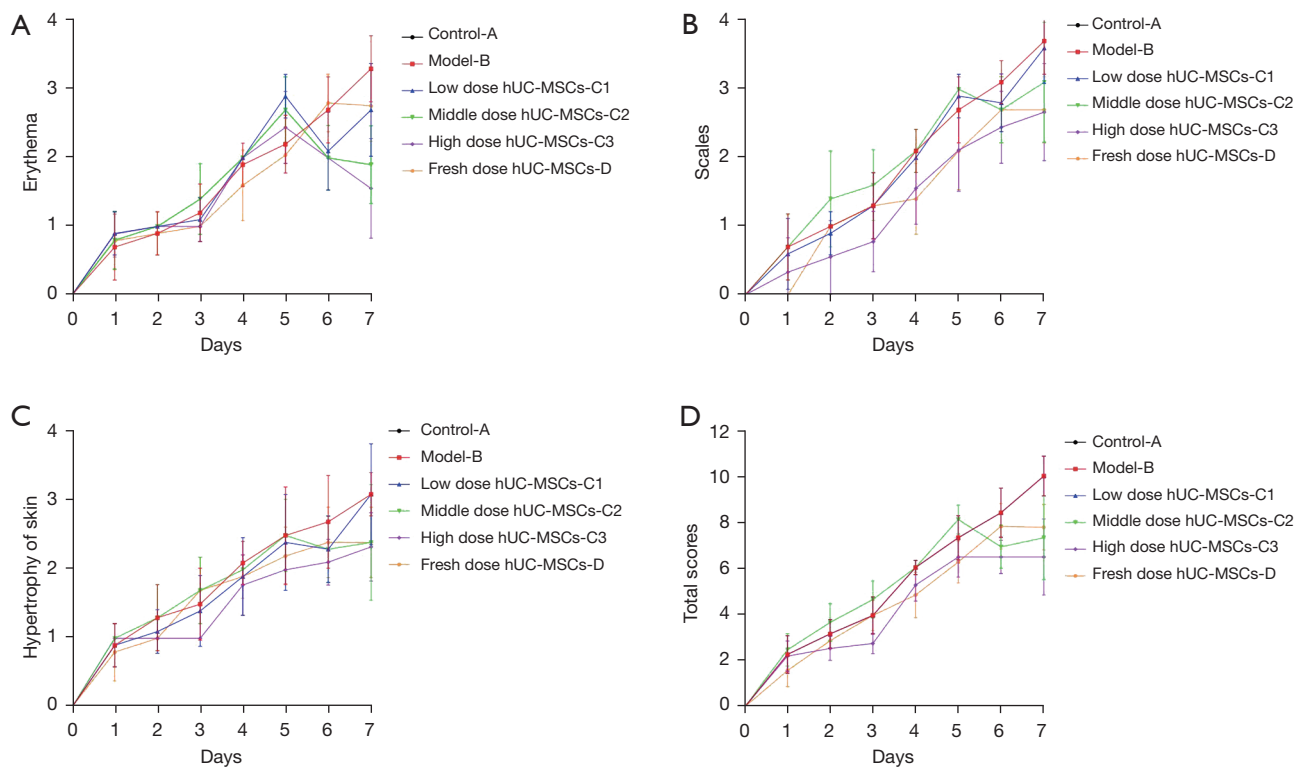
#### Data collection and statistical analysis

We used statistical software SPSS13.0 to process the data, and the measurement data are expressed as the mean ± standard deviation.

## Results

#### General presentation and skin lesion scores

In the experimental group, from the first day of modeling, minor psoriatic lesions appeared on the backs of the mice. Correspondingly, the PASI score increased day by day, and the overall trend of increase was obvious in each group. In the first 4 days, there was no significant difference in the PASI scores among the groups. On day 5, the symptoms of psoriasis in other groups were significantly ameliorated, the scores decreased, and the skin lesions began to improve. The scores of the psoriatic lesions and PASI in model group B and low-dose group C1 gradually became higher than those in the other groups, while the PASI scores in the medium-dose and high-dose groups C1 and C2 began to stabilize and remained at a lower level. On the 7th day of medication, there was a good dose-dependent relationship among the high-dose, medium-dose and low-dose groups. Compared with the model group and C1 group, psoriatic symptoms in the other groups were significantly reduced (Figure 1). Group A was the normal control group and did not participate in the scoring.



**Figure 1** PASI scores of animals in each group: (A) erythema score, (B) scaliness score, (C) skin hypertrophy score, (D) total PASI scores of animals in each group. PASI, Psoriasis Area and Severity Index.

### Changes in dorsal skin lesions

Changes in the dorsal skin lesions were observed by the naked eye (Figure 2). At 1 day after modeling, erythema appeared in each group and after 3–4 days, there was obvious scale formation. On the 5th day, the scaly erythema began to increase, and spotty bleeding was observed after the scales fell off. On day 6, the erythema of model groups B and C1 was aggravated, the skin was obviously thickened, surface scaliness continued to increase, and the skin rash was similar in the appearance to human psoriatic lesions. However, from day 5, erythema, scaliness and infiltration in all treatment groups except living group D were significantly less than in model groups B and treatment C1.

### Spleen index results

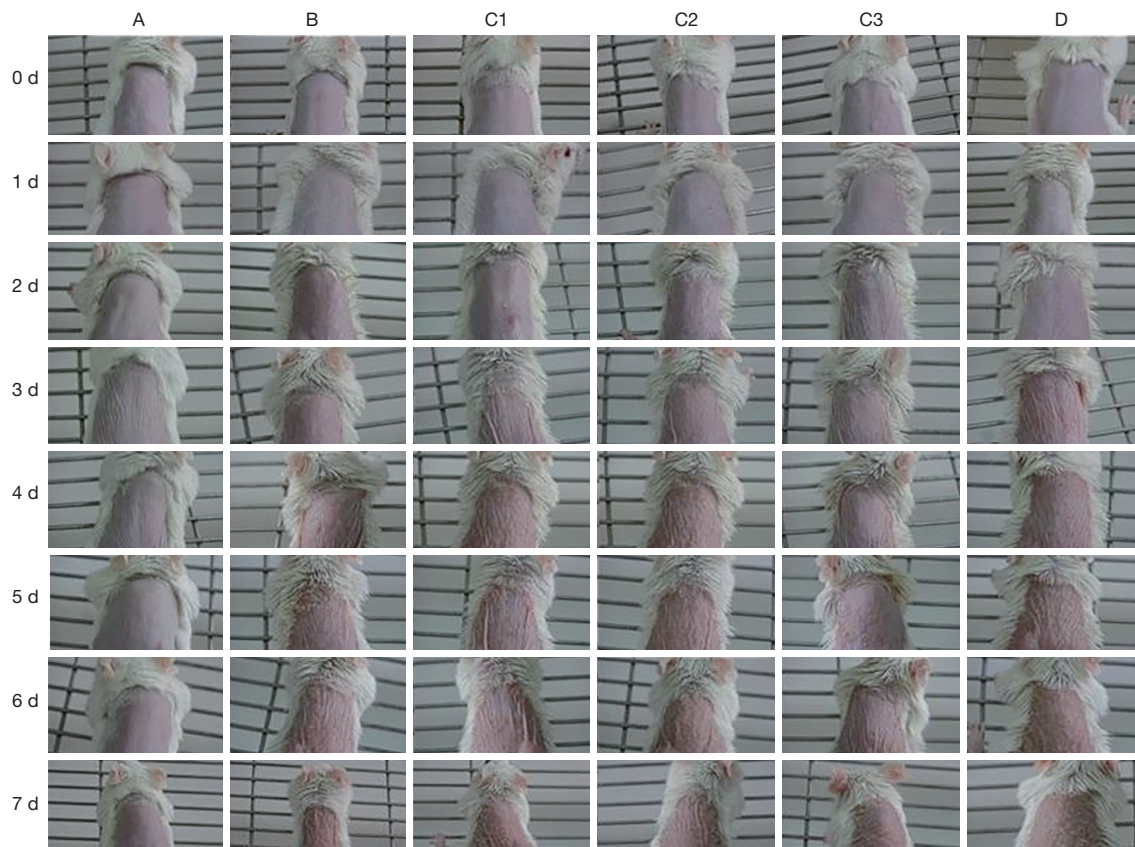
At the end of the experiment, the spleens of the animals in each group was measured and the spleen index (spleen index = spleen mass/body mass) was calculated (Figure 3). Both the spleen size and spleen index of mice in each group were significantly higher than those in control group A.

There was no significant difference in spleen index between the model groups B and the C1. The spleen index of the C1, C2 and C3 groups increased with increasing stem cell concentration.

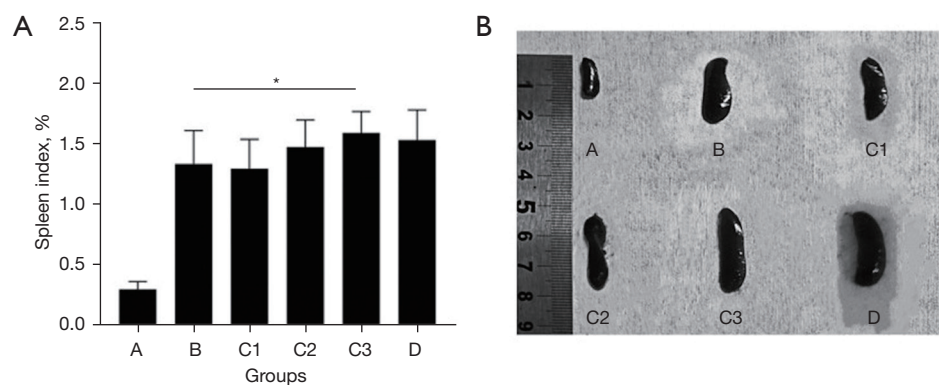
### Pathologic results

#### Symptoms observed

Observing the dorsal skin of the mice in each group, it could be seen that the epidermal layer of the mice in control group A was thinner, and only 1–2 layers of cells with normal morphology could be seen. Other groups showed obvious epidermal thickening that was greater than that of normal skin. There were Munro microabscesses in some locations, increased numbers of cells in the spinous layer, and a large number of inflammatory cells infiltrated the dermis, similar to the pathological changes of human psoriatic lesions. Skin lesions combined with pathological features proved that the imiquimod cream + LPS/IL-12 induced psoriasis model was successful. Compared with model group B, the number of parakeratosis cells in



**Figure 2** Real-time images of animals in each group after 0–7 days.

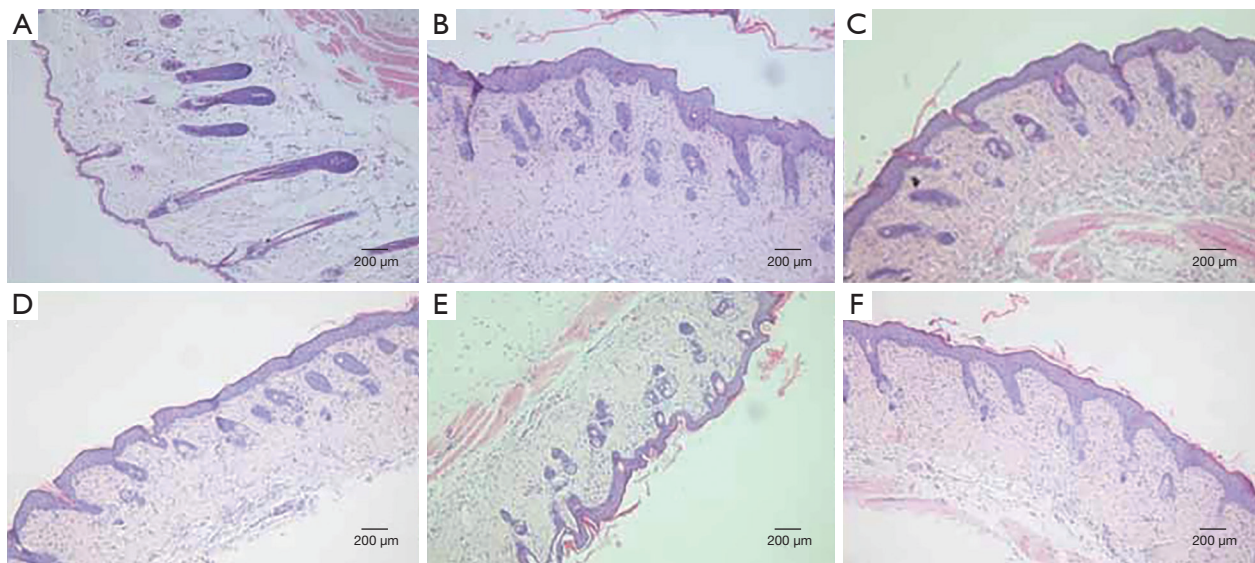


**Figure 3** Comparative spleen index (A) and spleen size (B). \*,  $P < 0.05$  indicates a significant difference compared with group A.

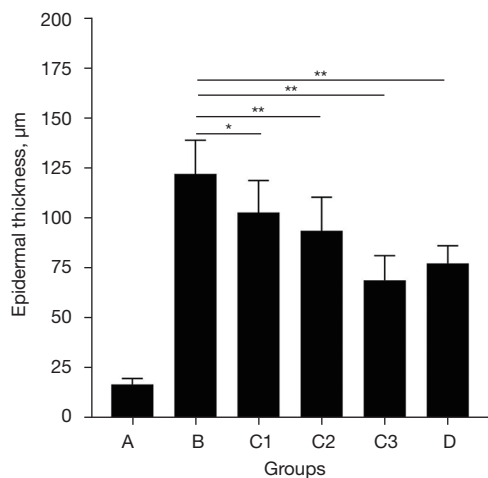
each group was significantly reduced, and the degree of dermal hyperplasia and inflammatory cell infiltration of erythrocytic extravasation was significantly lower than in model group B, and the C3 effect was the most obvious in the high-concentration hUC-MSCs group (Figure 4).

#### Skin measurement results

The epidermal thickness of each group was measured: B ( $122 \pm 17.0 \mu\text{m}$ ) > C1 ( $103 \pm 16.0 \mu\text{m}$ ) > C2 ( $94 \pm 17.0 \mu\text{m}$ ) > D ( $78 \pm 8.7 \mu\text{m}$ ) > C3 ( $69 \pm 12.4 \mu\text{m}$ ) (Figure 5). In addition to C1 group ( $0.01 < P < 0.05$ ), there were statistically significant



**Figure 4** Pathological changes of skin tissue of mice in each group ( $\times 200$ , HE). (A) Control group. (B) Model group. (C-E) Treatment C1, C2 and C3 group. (F) Fresh D group.



**Figure 5** Comparison of epidermal thickness in each group. \*,  $P < 0.05$  indicates a significant difference compared with group B; \*\*,  $P < 0.01$  indicates a significant difference compared with group B.

differences between each treatment group and model group B ( $P < 0.01$ ), and there were statistically significant differences in the other groups ( $P < 0.01$ ).

#### ELISA test results

The concentrations of *Tb1*- and *Tb17*-related immune molecules *TNF- $\alpha$* , *IFN- $\gamma$* , *IL-17A* and *IL-23* in the serum and skin of mice in each group were detected by ELISA

(Tables 2,3; Figures 6,7). We found that the expression concentrations of all cytokines in model group B were higher than those in the other groups. Among them, the expressions of *IFN- $\gamma$*  and *IL-17a* in the serum and skin of treatment group C1 were significantly different from that of model group B, but the expressions of *TNF- $\alpha$*  and *IL-23* were not significantly different from that of model group B. Excepting treatment group C1, the concentrations of the four factors in the serum and skin of the other groups were significantly different from those of model group B. Among them, the concentrations of the four cytokines in the serum and skin of the mice administered stem cell preparations with different concentrations were also different, and there was a certain negative correlation. In other words, the higher the dose concentration of the stem cell preparation, the lower the concentration of the four cytokines in serum and skin. These results suggested that the concentration of stem cells had an effect on the cytokine content in the serum and skin of the psoriatic mice.

#### Immunohistochemical results

The expression of *IL-36RN* protein was detected by immunohistochemistry, and the results showed that its expression level in the hUC-MSCs groups with different concentrations was lower than that in the control group A. However, the expression level of *IL-36RN* protein in the

**Table 2** Concentrations of cytokines in the serum of mice as determined by ELISA

Group	IFN- $\gamma$	TNF- $\alpha$	IL-17A	IL-23
Control A	369.3 $\pm$ 77.2**	378.8 $\pm$ 57.1**	17.6 $\pm$ 2.9**	44.6 $\pm$ 8.1**
Model B	666.5 $\pm$ 85.8	605.9 $\pm$ 64.6	29.8 $\pm$ 2.6	72.8 $\pm$ 8.0
Treatment C1	579.4 $\pm$ 97.7*	551.0 $\pm$ 65.6	25.6 $\pm$ 3.2**	68.2 $\pm$ 5.4
Treatment C2	531.7 $\pm$ 80.7**	488.6 $\pm$ 74.0**	23.9 $\pm$ 1.5**	58.1 $\pm$ 5.7**
Treatment C3	506.3 $\pm$ 66.8**	470.3 $\pm$ 38.3**	22.4 $\pm$ 3.0**	57.5 $\pm$ 7.5**
Fresh D	413.8 $\pm$ 58.2**	416.7 $\pm$ 57.5**	19.2 $\pm$ 2.7**	50.2 $\pm$ 6.4**

Serum concentrations of IFN- $\gamma$ , TNF- $\alpha$ , IL-17A and IL-23 were measured by ELISA on the 7th day after the death of each group of mice, and the data are the mean  $\pm$  labeling difference of 3 independent experiments ( $\bar{x}\pm s$ , n=10). \*, P<0.05 indicates a significant difference compared with group B; \*\*, P<0.01 indicates a very significant difference compared with model group B. ELISA, enzyme-linked immunosorbent assay; IFN, interferon; TNF, tumor-necrosis factor; IL, interleukin.

**Table 3** Concentrations of cytokines in skin tissue as determined by ELISA

Group	IFN- $\gamma$	TNF- $\alpha$	IL-17A	IL-23
Control A	3,688.5 $\pm$ 649.4**	4,134 $\pm$ 326.9**	184 $\pm$ 31.3**	408.6 $\pm$ 57.5**
Model B	5,997 $\pm$ 711.4	5,602.2 $\pm$ 489.4	258.1 $\pm$ 22.5	633.9 $\pm$ 69.4
Treatment C1	5,757.6 $\pm$ 742.7	5,028.2 $\pm$ 544.7**	252.2 $\pm$ 30.9	576.9 $\pm$ 34.4**
Treatment C2	5,101.7 $\pm$ 837.7**	4,531.9 $\pm$ 358.3**	248.5 $\pm$ 34.2**	580.3 $\pm$ 69.1**
Treatment C3	4,752.5 $\pm$ 658.1**	4,571.3 $\pm$ 643.3**	227.9 $\pm$ 33.0**	546.9 $\pm$ 89.1**
Fresh D	3,869.9 $\pm$ 840.1**	4,289.2 $\pm$ 542.9**	203.7 $\pm$ 28.1**	458.9 $\pm$ 71.4**

Concentrations of IFN- $\gamma$ , TNF- $\alpha$ , IL-17A and IL-23 in the skin of each group were measured by ELISA on the 7th day after the death of each group of mice, and the data are the mean  $\pm$  labeling difference of 3 independent experiments ( $\bar{x}\pm s$ , n=10). \*\*, P<0.01 indicates a very significant difference compared with model group B. ELISA, enzyme-linked immunosorbent assay; IFN, interferon; TNF, tumor-necrosis factor; IL, interleukin.

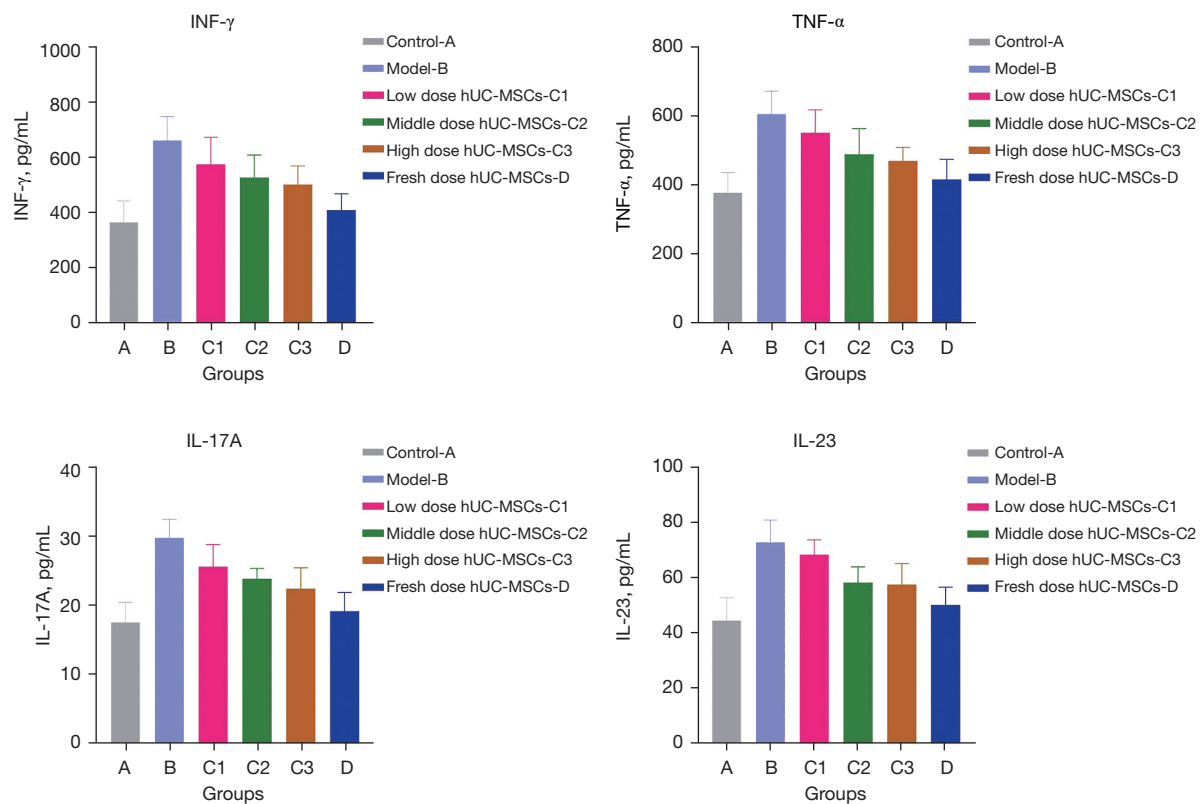
model group B was significantly lower than that in the other groups. Moreover, in the hUC-MSCs groups with different concentrations, the expression of IL-36RN protein showed an upward trend with increasing hUC-MSC concentration, and the two were positively correlated, which suggested that the administration of hUC-MSCs was related to the expression level of *IL-36RN*.

## Discussion

Psoriasis is a chronic inflammatory skin disease with a worldwide incidence of 2–3% (18). The immunological characteristics of psoriasis are immune cell infiltration, hyperkeratosis and parakeratosis, and dilatation of blood vessels in the dermal papilla (19). Psoriasis is mainly classified as erythematous plaque type, spotty type, reverse type, pustular type or erythroderma type (20). The

purpose of this study was to evaluate the pharmacodynamics and immune mechanism of hUC-MSCs at different concentrations in delaying psoriatic lesions in an *in vivo* experimental study in BALB/c mice. In mice with psoriatic lesions induced by imiquimod, the lesion severity peaked on days 5–8 and gradually subsided spontaneously due to differences in immune genes between mice and humans. Therefore, in order to ensure that the psoriatic characteristics of the mouse model were maintained for a longer time, in order to observe the efficacy of stem cell therapy, we established an improved psoriasis mouse model according to the method of Xiu *et al.* (21). After imiquimod cream was applied to the skin, *IL-12* was added to stimulate the immune system of mice to prolong the duration of skin lesions in the model, making the characteristics of psoriasis more typical, which improves the evaluation of the therapeutic effect of drugs. In addition, given that the





**Figure 6** Concentrations of cytokines IFN- $\gamma$ , TNF- $\alpha$ , IL-17A and IL-23 in the serum of mice in each group. IFN, interferon; TNF, tumor-necrosis factor; IL, interleukin; hUC-MSC, human umbilical cord mesenchymal stem cell.

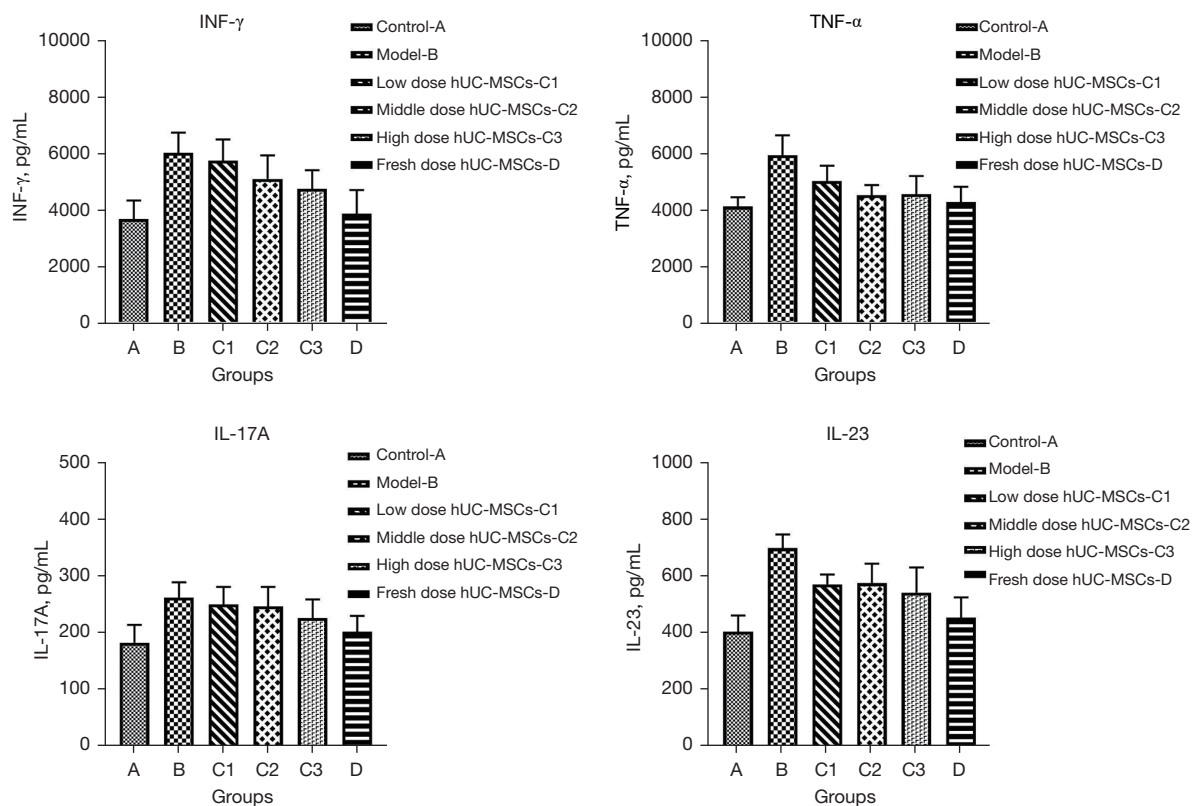
psoriatic mouse model has a tendency to self-healing, this study was designed to administer the drug before modeling to reduce the effect of stem cells due to the immune factors of the mouse model itself.

The experimental results showed that treatment with hUC-MSCs at different concentrations or with fresh hUC-MSCs before modeling led to a slow regression of the psoriatic lesions and a slow decrease in PASI score in model group B and low-dose group C1, while the PASI scores in the medium and high-dose groups C1 and C2 became stable and were maintained at a low level. On the 7th day after treatment, the dose of the high-, medium- and low-dose groups was inversely proportional to the curative effect. Moreover, the epidermal thickness was inversely proportional to stem cell concentration. With increasing hUC-MSCs solution concentration, the number of keratinocytes in the experimental groups was significantly reduced, and both the degree of erythrocytic extravasation dermal hyperplasia and inflammatory cell infiltration were reduced, suggesting that hUC-MSCs had therapeutic and

interventional effects on the psoriatic lesions in mice, and showed a good dose-dependent relationship.

The spleen size and spleen index of the experimental groups were significantly higher than that of control group A, and the comparison between experimental groups showed that the spleen index increased with increasing of stem cell concentration, suggesting that the spleen may be involved in the immune regulation process of inflammation in mice.

The immune system is involved in the whole process of the occurrence and development of psoriasis. Abnormal activation and infiltration of T lymphocytes in the epidermis or dermis are important pathophysiological features (22,23). T lymphocytes receive dendritic cells and other antigen-presenting cells to produce *IL-23*, *TNF- $\alpha$*  induces differentiation and proliferation of CD4+ T lymphocytes (Th17 cells), and mature Th17 cells can secrete various cytokines such as *IL-17*, *IL-21*, *IL-22*, etc., which keratinocyte overproliferation or inflammation of synovial cells. Therefore, *Th17* cells and the *IL-23/IL-17*



**Figure 7** Concentrations of cytokines IFN- $\gamma$ , TNF- $\alpha$ , IL-17A and IL-23 in the dorsal skin tissue of mice in each group. IFN, interferon; TNF, tumor-necrosis factor; IL, interleukin; hUC-MSC, human umbilical cord mesenchymal stem cell.

axis may be a key link in the pathogenesis of psoriasis and become a new therapeutic target (24). *IL-17* and *IL-12* are important cytokines in the *IL-23/IL-17* axis and *Tb1/Tb2* balance, which are two pathogenic pathways of psoriasis, and there are many biological agents targeting these two cytokines for the treatment of psoriasis (25-27). Therefore, *IL-23* and *IL-17* were selected as therapeutic targets in this study.

Therefore, the results of this study suggest that hUC-MSCs have a therapeutic effect on imiquimod-induced psoriasis in mice by inhibiting the concentration of *IL-23* and *IL-17* factors. And the higher the concentration of hUC-MSCs, the better the treatment effect.

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## Footnote

**Reporting Checklist:** The authors have completed the ARRIVE reporting checklist. Available at <https://atm.amegroups.com/article/view/10.21037/atm-22-4/rc>

**Data Sharing Statement:** Available at <https://atm.amegroups.com/article/view/10.21037/atm-22-4/dss>

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