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Dendritic cells modified with Der p1 antigen as a therapeutic potential for allergic rhinitis in a murine model via regulatory effects on IL-4, IL-10 and IL-13

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ABSTRACT

Objectives: House dust mites, including Der p1, are common allergens. The current study was designed to explore the allergen-specific immune tolerance effects of Der p1-modified dendritic cells (DCs) through IL-4, IL-10 and IL-13 on an allergic rhinitis (AR) mouse model.

Methods: A lentivirus was modified to express *Derp1*. Then, immature DCs from mice were infected with this modified lentivirus to generate a lenti-*Derp1*-GFP DCs. 24 mice were random divided into four groups (n = 6 each), AR mouse were sensitized by *Derp1* allergens and treated with lenti-GFP DCs (GFP-DC/AR group), or lenti-*Derp1*-GFP DCs (Der p1-DC/AR group) and dexamethasone (Dex/AR group), mice in the control group were treated with PBS instead of Der p1 then also intraperitoneally injected with 5×10^6 lenti-GFP DCs/mouse. AR symptoms expressed by each mouse were recorded. The proportions of $CD4^+CD25^+Foxp3^+$ regulatory T cells among $CD4^+$ T cells in the peripheral blood, and mRNA and protein expression levels of IL-4, IL-10, and IL-13 were measured.

Results: DCs infected with lenti-*Derp1*-GFP stimulated the maturation of DCs. Compared with the GFP-DC/AR group, mice in the Der p1-DC/AR group showed an ameliorated allergic response, a significant decrease in the levels of serum IgE, IgG1, and histamine, and a decrease in the expression of IL-4 and IL-13 mRNA and protein in the nasal mucosa. The expression of IL-10 increased in the Der p1-DC/AR group to a level similar to that observed in the Dex/AR group.

Conclusions: These results indicate that Der p1-modified DCs have therapeutic potential for AR via down-regulation of IL-4 and IL-13, and upregulation of IL-10.

1. Introduction

Gene-modified immune cells have been an area of recent research interest. Dendritic cells (DCs) are immune cells with unique characteristics that make them ideal for gene modification. They are considered the most effective antigen-presenting cell (APC) responsible primarily for sensitization of naive T cells to specific antigens. DC therapy (DC vaccines) represents a new and promising immunotherapeutic approach to treat allergic diseases, as well as to enhance antigen recognition and presentation through transfection of antigen genes [1,2].

For allergic diseases of the airway, such as allergic rhinitis (AR) and asthma, morbidity is high and the pathogenesis is complex. Despite its significant progress, conventional immunotherapy using protein-based vaccines has shown to be still time-consuming and cumbersome. In recent studies, several strategies to minimize allergenicity or enhance immunogenicity have been attempted to improve efficiency [3]. Progress in DC vaccines has made this possible. Increased antigen expression can induce immune tolerance in vivo; therefore, exploration of DC vaccines against allergens is a new direction for research and development for allergic diseases of the airway [4].

Allergen-specific immunotherapy has been shown to induce allergen-specific immune tolerance, most likely through the upregulation of IL-10 and downregulation of IL-4 and IL-13 [5]. IL-4 and IL-13 are immunoregulatory cytokines that are predominantly secreted by activated Th2 cells. IL-4 and IL-13 are recognized as key mediators of AR pathogenesis, and they directly influence Th2 cell maturation and migration [6,7]. IL-10 has immunosuppressive functions and is an important factor in inducing immune tolerance in patients with allergic airway inflammation [8].

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Der p1, an allergen from the house dust mite *Dermatophagoides pteronyssinus*, is one of the most thoroughly-studied and well-characterized allergens [9]. In a previous study, we constructed *Derp1* gene-modified DCs (*Derp1*-DC) and found that *Derp1*-DCs could increase the Th1:Th2 ratio and the percentage of $CD4^+CD25^+Foxp3^+$ regulatory T (Treg) cells in an AR murine model [10]. Immunotherapy by *Derp1*-DC can induce immune tolerance and relieve clinical symptoms; however, the underlying mechanism is not thoroughly understood. To investigate the therapeutic mechanisms of *Derp1*-DC, their effects on IL-4, IL-10, and IL-13 expression in the nasal mucosa were evaluated relative to a glucocorticoid treatment in a mouse model.

2. Materials and methods

2.1. Materials

The following materials were employed: pcDNA3.1(+) (Invitrogen, USA); BamHI, NheI, and GeneRuler DNA Ladder (Fermentas, Canada); T4 ligase (NEB, USA); anti-mouse FITC-CD11c, CD11b, CD59 and CD103 (eBioscience, USA); CD80 and CD86 antibody (Bioss Inc., China); male Balb\c mice (SLACCAS, Shanghai); Mission Lentiviral Packaging Mix (Invitrogen, USA); and Der p1 protein (ProSpec, USA). All animal care and experimental procedures were performed in accordance with the guidelines of the National Institutes of Health and were approved by the Tongji University Institution Animal Care and Use Committee (2013-DW-008).

2.2. Cloning, construction, and titration of a lentivirus-based vector expressing Der p1

The *Derp1* gene of dust mites was synthesized and then digested with NheI/BamHI and inserted into plasmid pcDNA3.1(+). The GFP gene was amplified from pEGFP by PCR using the forward primer 5′-TTC GGG ATC CGT GAG CAA GGG CGA GGA GC-3′ and reverse primer 5′-ATT ACT CGA GTT ACT TGT ACA GCT CGT CCA TGC C-3′. The gene was digested with BamHI/XhoI and cloned into pcDNA3.1(+)-*Derp1*. Then, the plasmids were transformed into *Escherichia coli* cells, isolated, and verified by DNA sequencing.

The gene encoding *Derp1*-GFP pCDNA3.1(+)-*Derp1*-GFP was amplified by PCR. Then, the PCR product was digested and cloned into lentivirus vector pLenti6.3-MCS/V5 DEST. Subsequently, this vector plasmid, constructed with *Derp1*-GFP, was transfected into 293 T cells using Mission Lentiviral Packaging Mix. Forty-eight hours after transfection, the constructed viral vector was harvested by multiple freeze-thaw cycles and purified with PEG. The generated lentivirus was named lenti-*Derp1*-GFP, and the titre of the virus was confirmed by limiting dilutions on 293 T cells.

2.3. DC isolation and culture

Healthy Balb/c mice were decapitated, and femurs and tibias were obtained through a sterile procedure. Muscles and remaining tissues were removed from bones, and each end of each bone was excised. A sterile syringe was used to fill the bone cavity with RPMI1640 medium and wash out the bone marrow; then, the bone marrow was collected by centrifugation. DCs were suspended and enriched in RPMI1640 supplemented with 10% fetal bovine serum (FBS) and 10 μg/L of granulocyte macrophage colony-stimulating factor (GM-CSF), and then incubated at 37 °C and 5% CO_2. The medium was changed every 3 days. On the 5th day, the cells, which were immature DCs, were collected by centrifugation.

2.4. Lenti-Derp1-GFP infection of DCs, flow cytometry, and immunofluorescence staining

DCs were infected with lenti-*D*erp1-GFP at an MOI of 100:1 in RPMI1640 with 20% FBS and 10 μg/L of GM-CSF. On the 10th day, mature DC infected by lenti-*Derp1*-GFP and lenti-GFP (called *Derp1*-DCs and GFP-DCs) and imDCs were analysed by flow cytometry using antibodies against markers of mature DCs, namely CD11c, CD11b, CD59, and CD103 (eBioscience Inc., USA), then conjugated with secondary antibodies labelled with Alexa Fluor488 (Bioss Inc., Beijing, China). Cell surfaces were stained following standard flow cytometry procedures.

DCs were stained to observe the expression of co-stimulatory molecules CD80 and CD86 by immunofluorescence microscopy. DCs were collected by centrifugation (1000 rpm for 5 min) and fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 15 min at room temperature (RT). Then, the cells were permeabilized with 0.2% Triton X-100 in PBS for 20 min at RT and blocked with 5% BSA in PBS for at least 1 h at RT. Rabbit polyclonal primary antibody was diluted 200-fold in PBS and incubated with the cells for overnight at 4 °C, followed by five washes with PBS. The cells were then incubated with mouse anti-rabbit IgG/Cy3 secondary antibody. Finally, the cells were washed, stained with DAPI (Vectashield) for cell nucleus detection, and dropped onto slides for microscopic observation. The slides were examined on a LSM780 laser-scanning microscope (Nikon C2 Plus, Japan).

2.5. Mixed leukocyte reaction (MLR) analysis

The ability of *Derp1*-DC to stimulate naive T cells, was determined by MLR. Various numbers (2×10^3–2×10^5/well) of *Derp1*-DC, imDC and GFP-DC, pretreated with mitomycin C (25 μg/mL, Amresco, USA), were cocultured with splenic T-cells from Balb/c mice as responders (2×10^5/well) at three different ratios of responder cells to stimulator cells (5:1, 10:1, 20:1) for 96 h in 0.2 mL. RPMI-1640 complete medium in a 96-well U-bottomed microtiter plate. The proliferation of T cells was determined by Flow cytometry, using CFSE staining kit (Invitrogen, USA).

2.6. Sensitization, airway challenge, and treatment of an AR model

AR animal models were prepared according to the method proposed by Akdis et al. [5]. Purified natural Der p1 allergens (Indoor Biotechnologies Inc., USA) were used for model sensitization. Twenty-four male mice were randomly divided into four groups, with six mice in each group. AR mouse models were sensitized by subcutaneous injection with 1.5 μg Der p1 per mouse in PBS on days 1, 3, 5, 8, and 10, and by intraperitoneal injection on days 12, 15, and 17; and by nasal inhalation on days 19, 22, and 24. From Day 24, intranasal challenge was carried out with Der p1 via intranasal instillation for 6 consecutive days to maintain AR symptoms. Mice in the control group were treated with PBS instead of Der p1 at each time point. Frequencies of scratching and sneezing were assessed as previously described by Suleimani [11].

Then, the sensitized mice were treated with 5×10^6 lenti-GFP DCs/mouse (GFP-DC/AR group, intraperitoneal injection), 5×10^6 lenti-*Derp1*-GFP DCs/mouse (Der p1-DC/AR group, intraperitoneal injection), and 2 mg/kg of dexamethasone (Dex/AR group, intraperitoneal injection). The control mice were also intraperitoneally injected with 5×10^6 lenti-GFP DCs/mouse. All mice received the treatments twice a week, for four weeks. Two hours after the last treatment, nasal symptoms (i.e., scratching and sneezing) were observed for 10 min. Sneezing was characterized by an explosive expiration immediately following deep inspiration. Scratching was characterized by an external perinasal scratch with an animal's forelimb.

Nasal lavage fluid (NLF) was collected from the anterior naris by washing nasal cavities with 2 mL of prewarmed saline infused from the tracheal side. NLF was centrifuged and precipitated, then was suspended with 0.5 mL Hinkelmann solution. 20 μL suspension was mixed with 380 μL ethanol-eosin diluent, then filled into the cell count pool and the eosinophils were counted [12]. Nasal mucosa were fixed in 4%

Table 1
Sequences of primers used for real-time quantitative polymerase chain reaction.

Gene	Sequences (5′ → 3′)	Product length (bp)
GAPDH	F-GGCAAATTCAACGGCACAGT	255
	R-CTCGTGGTTCACACCCATCA	
IL-4	F-AACGTCCTCACAGCAACGAA	164
	R-AGGCATCGAAAAGCCCGAAA	
IL-10	F-CAGTACAGCCGGGAAGACAA	229
	R-CCTGGGGCATCACTTCTACC	
IL-13	F-CTTGCTTGCCTTGGTGGTCT	122
	R-CACAGGGGAGTCTGGTCTTG	

formaldehyde, embedded in paraffin, and sectioned (4 μm), followed by H&E staining for histological observation.

2.7. Enzyme-linked immunosorbent assay (ELISA) of histamine, IgG1, and IgE in mouse serum

All mice were sacrificed 2 h after the last treatment. Sera were isolated from blood samples following eyeball extirpation and stored at −80 °C. Total IgG1 (Thermo Scientific Pierce Inc., USA), IgE, and histamine (Market Design Inc., USA) levels were measured by ELISA according to the manufacturer's instructions.

2.8. Quantitative real-time PCR (RT-PCR) of IL-4, IL-10, and IL-13

Total RNA from the nasal mucosa was extracted, reverse transcribed into cDNA, and amplified to determine fluorescence according to the manufacturer's instructions. IL-4, IL-10, IL-13, and GAPDH were detected. GAPDH was utilized as the housekeeping gene. Primer sequences are listed in Table 1. PCR was performed as follows: denaturation at 95 °C for 15 s, 40 cycles of 95 °C for 15 s, 56 °C for 30 s, and 72 °C for 32 s. Relative gene expression was analysed by the $2^{-\Delta\Delta Ct}$ method.

2.9. Western blot analysis and immunofluorescence assay of IL-4, IL-10, and IL-13

Total protein content was measured by Bradford assay. Equal amounts of protein were electrophoresed on a 10%-sodium dodecyl sulphate (SDS) gel and then transferred to a polyvinylidene fluoride (PVDF) membrane. The membranes were blocked and then incubated for 4 h with primary anti-IL-4, anti-IL-10, and anti-IL-13 antibodies at a 1:1000 dilution (Santa Cruz, USA) overnight at RT. After this, the membranes were washed and incubated with secondary antibodies for 2 h at 4 °C. GAPDH was used as an endogenous control for protein normalization. The blots were visualized using chemiluminescence detection reagents. The experiment was performed in triplicate.

Nasal mucosal tissues were fixed in 10% buffered formalin. The tissue sections were deparaffinized and permeabilized in precooled acetone at −20 °C for 15 min. The slides were incubated for 1.5 h at RT with anti-IL-4 rat monoclonal antibody (1:100, Boster Co.BA0980-1, Ltd., China). Slides were washed twice in PBS, incubated for 30 min at RT with goat anti-rabbit IL-4 IgG/Cy3 (1:500, Boaoson, bs-0295G-Cy3, China). Cells were observed and photographed under a fluorescence. The method for detecting IL-10 and IL-13 was the same as that for IL-4.

2.10. Flow cytometric analysis

The proportion of Treg cells was determined by flow cytometric analysis. Briefly, a single cell suspension of peripheral blood mononuclear cells (PBMCs) from peripheral veins was labelled with anti-CD4-fluorescein isothiocyanate (FITC) and anti-CD25-allophycocyanin (APC) monoclonal antibodies (BD, USA). For intracellular staining of Foxp3, cells were fixed and permeabilized using a Human Foxp3 Buffer Set according to the manufacturer's instruction (BD, USA). Non-specific fluorescence was determined using isotype-matched IgG as a control. Data were acquired using a FACS Calibur instrument (Beckman Coulter, USA) and analysed with CellQuest software (BD, USA).

2.11. Statistical analyses

All statistical analyses were performed using SPSS 19.0. Student's *t*-test and one-way analysis of variance (ANOVA) were utilized to determine differences with a p-value < 0.05. The data were visualized by as means ± standard deviations (SDs) for all indices.

3. Results

3.1. Amplification, purification, and titration of the lentivirus-based vector expressing Der p1

pcDNA3.1(+)-*Derp1* showed two bands at around 1700 bp and 5000 bp by agarose gel electrophoresis and double enzymatic digestion. The *Der p1* gene was approximately 1700 bp. After the green fluorescence protein (GFP) PCR product was ligased into pcDNA3.1(+)-*Derp1*, the resulting product was transformed and isolated. The fusion product of *Der p1* and *GFP* was then subjected to PCR and electrophoresis, which showed the correct band at approximately 4000 bp. Sequence alignment in BLAST showed that *Der p1* and *GFP* were successfully cloned into pcDNA 3.1(+). The lentiviral vectors carrying *Derp1-GFP* and *GFP* alone were successfully constructed, resulting in viral titres of 1.9×10^8 TU/mL and 2×10^8 TU/mL, respectively.

3.2. Characterization of Derp1-DC by fluorescence and western blot

After infection with the recombinant lentiviruses, the modified DCs were observed under a fluorescence microscope. As shown in Fig. 1A, green fluorescence indicated successful infection of DCs with the *Der p1* lentivirus. The infection efficiency was > 95%. As shown in Fig. 1B, Der p1 was detected to verify antigen expression in *Derp1*-DC, not in DCs infected with the lentivirus expressing GFP alone. All this indicated that *Der p1* was specifically transfected and expressed in DCs.

3.3. Characterization of DCs and T cell proliferative capacity

DCs were studied using monoclonal antibodies against maturation markers CD11c, CD11b, CD59, and CD103. The immature DCs infected with lenti-GFP expressed low amounts of maturation markers. In the case of *Derp1*-DC, an increase in the expression levels of the four maturation markers was observed (Fig. 2A). Maturation markers CD80 and CD86 were also used to determine the DC phenotype. DCs infected with lenti-GFP expressed low amounts of maturation markers, and their phenotype was that of immature DCs. Two maturation markers, CD80 and CD86 (Fig. 2B), were highly expressed and localized in the cytoplasms of *Derp1*-DC. These results indicated that DCs stimulated by lenti-*Derp1*-GFP expressed some maturation markers and probably corresponded to mature DCs.

Only mature DCs generally are potent inducers of T cell proliferation which has been recognized as the sign of increasing immune responses. DCs as stimulators were co-cultured with lymphocytes (as responders) in 3 ratios of 1/5, 1/10 and 1/20 (stimulator/responders). According to the results shown in Fig. 2C, DCs matured with *Der p1* significantly induced higher T cell proliferation than imDCs and GFP-DCs in 1/5 and 1/10 ratio ($p < 0.05$).

3.4. Scratching, sneezing, eosinophil infiltration

A schema of the experimental protocol for AR was shown as Fig. 3A. The frequencies of scratching, sneezing and the numbers of eosinophils in NLF of mice are shown in Fig. 3B and C. The frequencies of

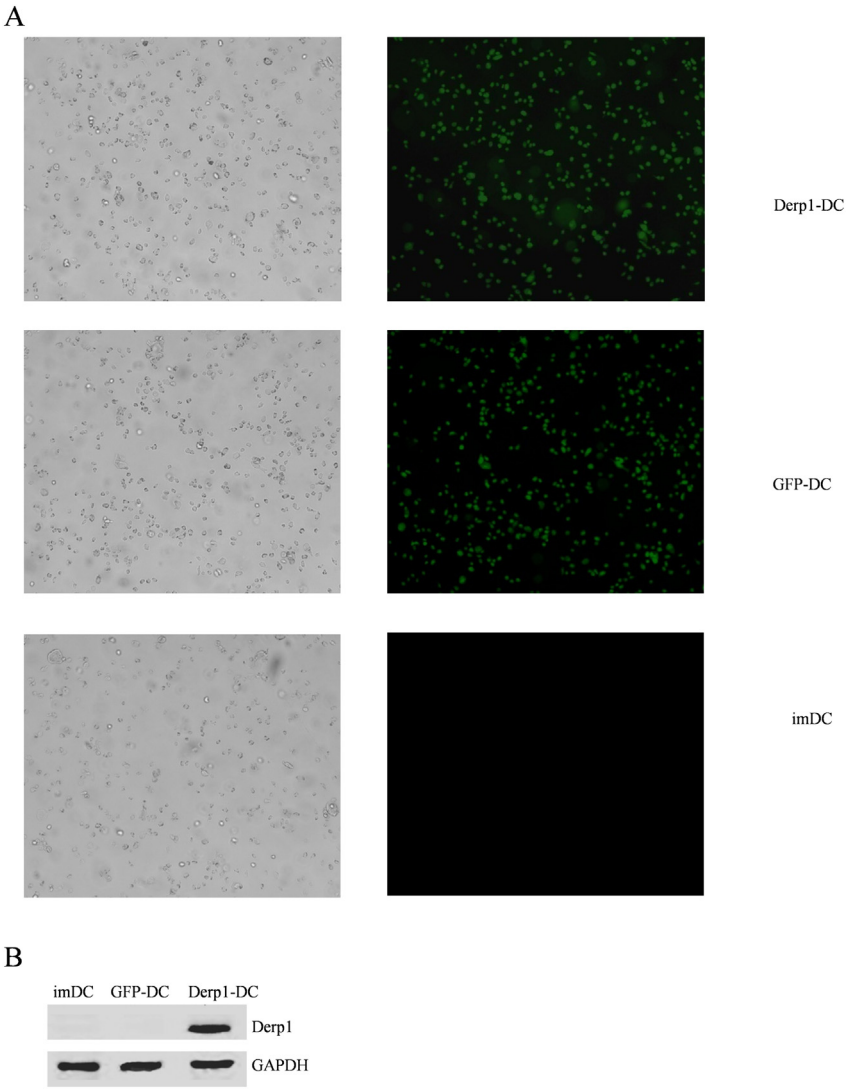

Fig. 1. Infection of DCs with green fluorescence protein (GFP)-expressing lentivirus to show infection efficiency. (A) GFP expression in fluorescence (left) and bright light (right) is shown. More than 90% of the cells were GFP positive in *Derp1*-DC and GFP/DC. (B) Der p1 was detected to verify antigen expression in *Derp1*-DCs. The results indicated Derp1 expressed in DCs signifying successfully infection using recombinant lenti-*Derp1-GFP* lentivirus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

scratching, sneezing and eosinophil infiltration in the GFP-DC/AR group were significantly increased compared with those of the control ($p < 0.05$). In the Dex/AR and *Derp1*-DC/AR groups, the frequencies of scratching, sneezing and eosinophil infiltration decreased significantly relative to those of the GFP-DC/AR group ($p < 0.05$). The indices of *Derp1*-DC/AR and Dex/AR mice were not significantly different. These results indicated that treatment with *Derp1*-DCs had a similar effect on inhibiting allergic inflammation in vivo as glucocorticoid therapy.

3.5. Serum levels of histamine, IgG1, and IgE and histological observations of nasal mucosa

Levels of histamine, IgG1, and IgE in mouse serum increased in the AR groups compared to the control group ($p < 0.05$), whereas mice administered *Derp1*-DC exhibited a decrease in these levels compared to those of the GFP-DC/AR group ($p < 0.05$) (Fig. 3D,E,F). Furthermore, there was no significant difference between mice in the *Derp1*-DC/AR and Dex/AR groups in terms of serum histamine, IgG1, and IgE levels. Histological observations indicated that a large amount of eosinophil infiltration was shown in nasal mucosa interstitial tissue of AR group, and less eosinophil infiltration was shown in the *Derp1*-DC/AR and Dex/AR groups (Fig. 4). These results indicate that *Derp1*-DC has the potential to relieve allergic inflammation.

3.6. Expression of IL-4, IL-10 and IL-13 mRNA and protein in the nasal mucosa

As shown in Figs. 5 and 6, the IL-4 and IL-13 mRNA and protein expression levels in the nasal mucosa were significantly increased in mice in the GFP-DC/AR group compared to those in the control group ($p < 0.05$). However, the mRNA and protein levels of IL-4 and IL-13 in the *Derp1*-DC/AR group were significantly lower than those in mice in the GFP-DC/AR group ($p < 0.05$). In addition, there was no significant difference in the expression of these mRNAs and proteins between mice in the *Derp1*-DC/AR and Dex/AR groups. However, the expression of IL-10 showed the opposite trends in the different groups. These results indicated *Derp1*-DC inhibited IL-4 and 13 expression but upregulated IL-10 expression during allergic inflammation.

3.7. Proportion of Treg cells of PBMCs

Results of the flow cytometric analysis of Treg cells from mice in the

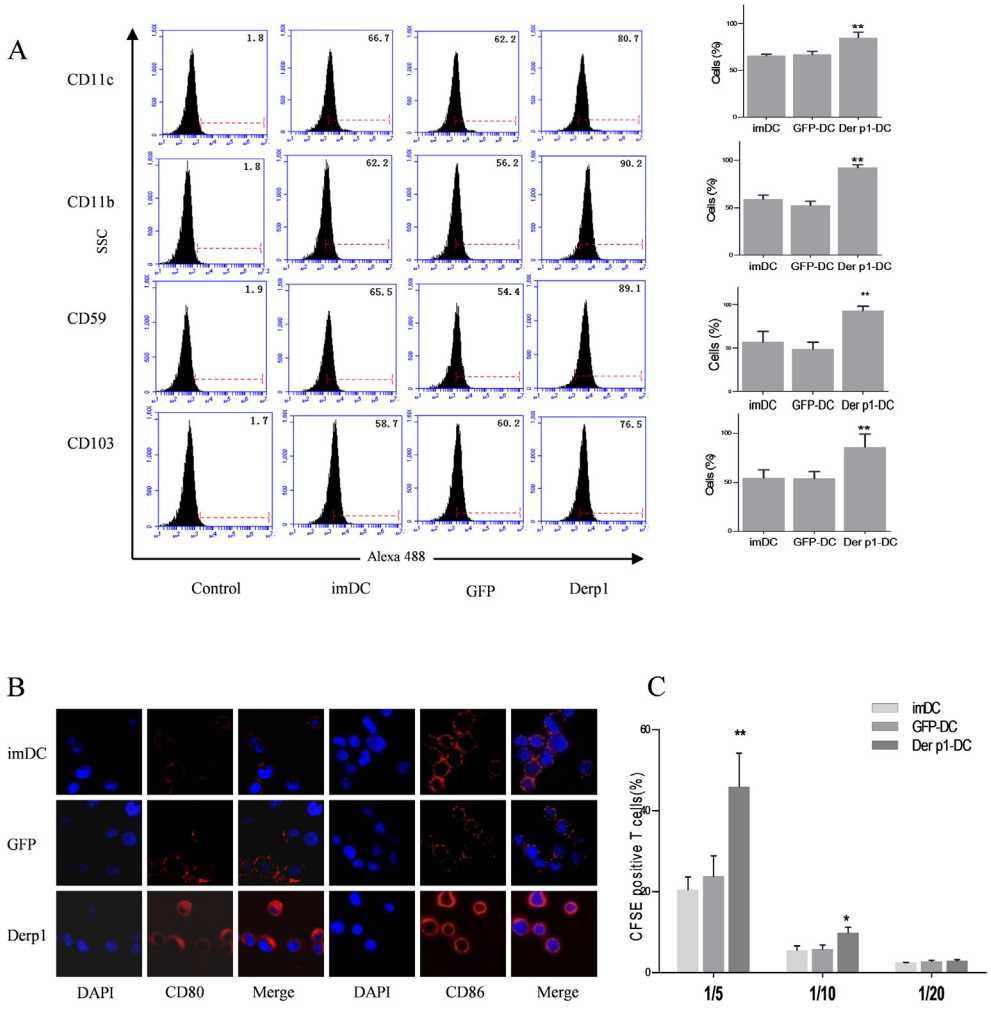

Fig. 2. Characteristics of *Der p1*-modified dendritic cells (DCs). (A) Surface expression of CD11c, CD11b, CD59, and CD103 by DCs after transfection with lenti-*GFP* and lenti-*Derp1*. DCs without any stimulation served as negative controls. DCs were stained with Alexa Fluor488 and were analysed by flow cytometry. Ten thousand cells were counted for each sample. Data are presented as the percentage of fluorescent cells among all DCs·(B)Immune staining showing CD80 and CD86 expression in DCs. Top: DCs without any stimulation; middle: DCs transfected with lenti-GFP; bottom: DCs transfected with lenti-*Derp1*. Left: CD80 or CD86; right: 4′,6-diamidino-2-phenylindole (DAPI); middle: merged DAPI + CD80 or CD86. CD80 and CD86 expression was observed in the cytoplasm of *Derp1*-DC (red). (C)*Derp1*-DC strongly induced proliferation in lymphocytes. This figure demonstrates the significantly higher potential of *Derp1*-DCs than imDCs and GFP-DCs to induce proliferation a production (in 1/5 and 1/10 ratio) from splenic cells. (ratio: Stimulator/responders).*, $p < 0.05$, means significantly different from imDCs. The experiment has been repeated for two times with consistent results. (original magnification × 400). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

different groups are shown in Fig. 7. The percentage of Treg cells in the AR group (0.54 ± 0.14%) was significantly lower than that in the control (7.25 ± 1.93%, $p < 0.01$), whereas that in the *Derp1*-DC/AR group was much higher (5.22 ± 1.53%) than that in the AR group ($p < 0.01$). However, the difference between the percentages of these cells in spleen tissues in mice in the *Derp1*-DC/AR and Dex/AR groups was not significant ($p > 0.05$).

4. Discussion

This study was conducted to determine the therapeutic effects of *Derp1*-DC on AR through the regulation of IL-4, IL-10, and IL-13 expression in a mouse model. The results showed that the *Derp1*-DC could relieve symptoms of AR inflammation and decrease serum levels of histamine, IgE, and IgG1, with effects similar to those of dexamethasone treatment. This research also indicated that *Derp1*-DC could inhibit the expression of IL-4 and IL-13 and upregulate IL-10 expression during allergic inflammation.

Specific immunotherapies for AR, an allergic disease, have been widely used clinically. Sublingual immunotherapy (SLIT) has been shown to be an effective method of allergen-specific immunotherapy in allergic disease. Its therapeutic effect is based on the ability of sublingual APCs to absorb antigens; however, sublingual DCs are not abundant, leading to a weak immune response and poor compliance in patients, requiring repetitive and long-term therapy (generally 2 years or more). Therefore, clinical application of SLIT is limited [13]. Improving antigen presentation is a pressing issue for immunotherapy.

Dust mite gene vaccines show stability, are easy to produce and store, can be produce with high purity, have strong immune effects, and result in long-lasting immunity; however, these vaccines can induce an inappropriate immune response. An excessive response may lead to self-reaction, which causes immunosuppression with a long-lasting high level of antigen in the body. Furthermore, immunotolerance can be induced with a low level of antigen, with the risk of DNA integration into the human genome [14]. In addition, low transgenic efficiency is an obstacle to its application. A weak immune effect accompanies low transgenic efficiency of a plasmid vector, whereas virus vectors present a safety risk. Therefore, an effective and safe immunotherapeutic approach is needed.

In recent years, immune cell vaccines have been a focus of research, with substantial attention devoted to the DC vaccines. The effective antigen presentation and strong immunity activation of DCs determine their complex immune regulatory abilities, including the ability to induce both tolerance and immunity. Therefore, DCs can not only present antigens, but they can also be used as immune vaccines [15]. Currently, DC vaccines are most often employed in tumour therapies. Wang et al. report that DCs can be sensitized by freeze-thawing tumour antigens derived from the human tongue cancer cell line Tca8113 [16]. In in vitro co-culture, sensitized DCs can activate cytotoxic T lymphocytes, which in turn enhance specific cytotoxicity against Tca8113 cells and inhibit cancer cell proliferation. Clinical trials have also shown that DC vaccines are a safe and effective strategy to induce T cell immune responses against tumours [17]. The first DC-based cancer vaccine, sipuleucel-T, was approved by the US Food and Drug Administration (US FDA) to treat metastatic prostate cancer [18].

In recent years, DC vaccines for AR and asthma have been

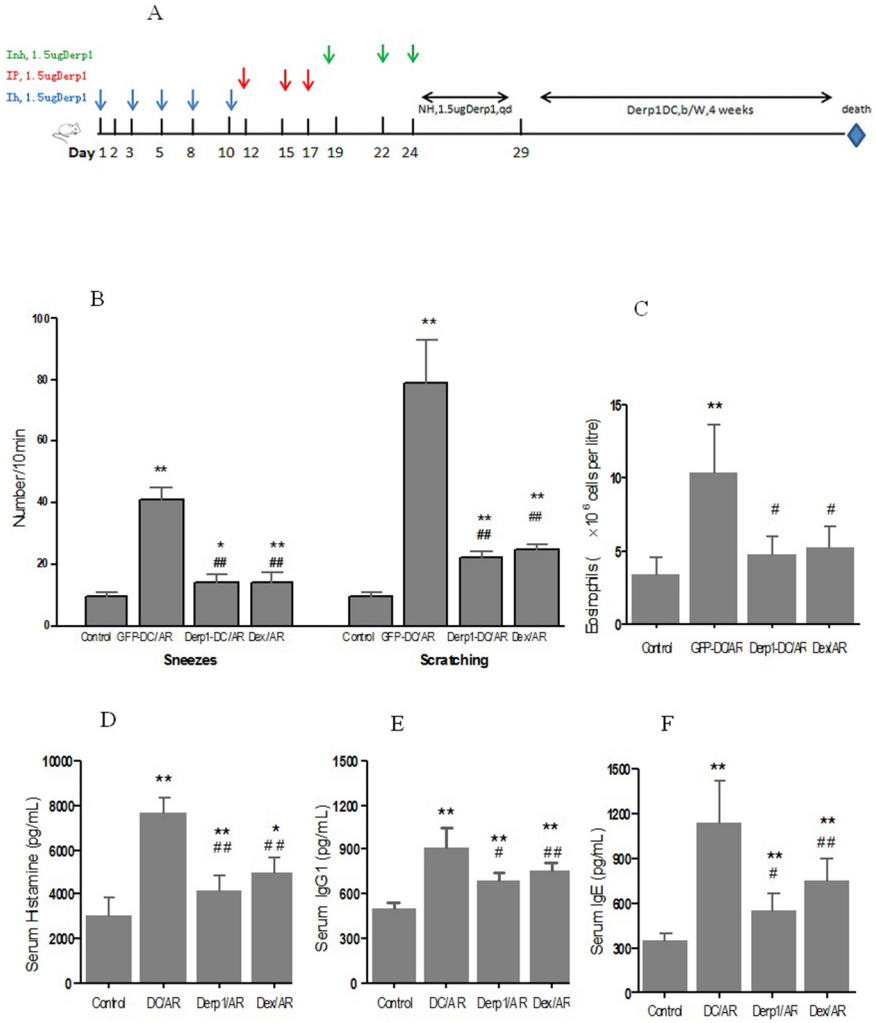

Fig. 3. Parameters of inflammation in the different groups. (A) A schema of the experimental protocol for AR. (B) Frequencies of scratching, sneezing. (C) Numbers of eosinophils in the nasal lavage fluid(NLF). (D) Serum levels of histamine. (E) Serum levels of IgG1. (F) Serum levels of IgE. Each column and vertical bar represents the mean ± SD. *, **: Significantly different from the control group ($p < 0.05$ and $p < 0.01$, respectively); #, ##: Significantly different from the AR group ($p < 0.05$ and $p < 0.01$, respectively).

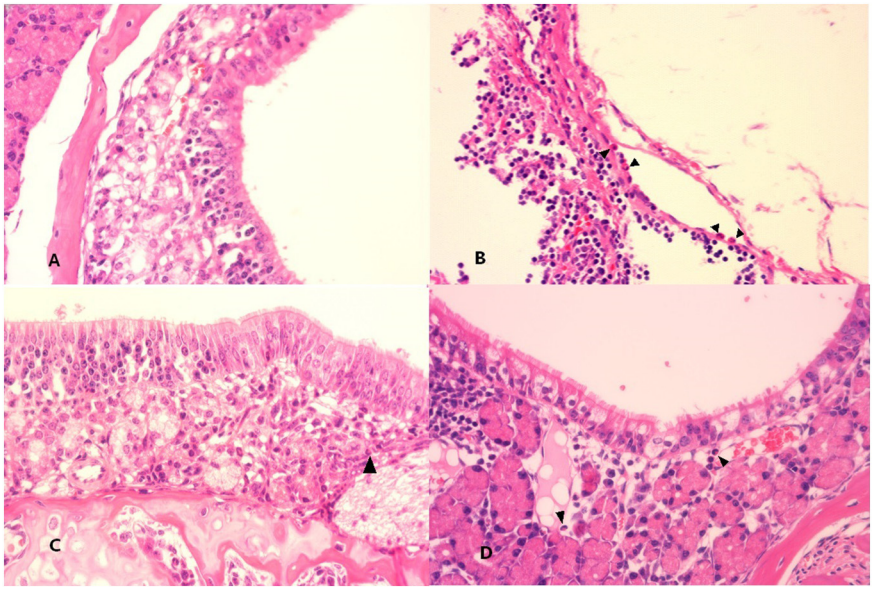

Fig. 4. Histological observation of nasal mucosa (H&E staining, magnification × 400). No eosinophil cells infiltration was shown in control group (A) and a large amount of eosinophil cells infiltration was shown in nasal mucosa interstitial tissue of GFP-DC/AR group (B), and less eosinophil cells infiltration was shown in Dex/AR group (C) and *Derp1*-DC/AR therapy groups (D). Arrows represent eosinophil cells.

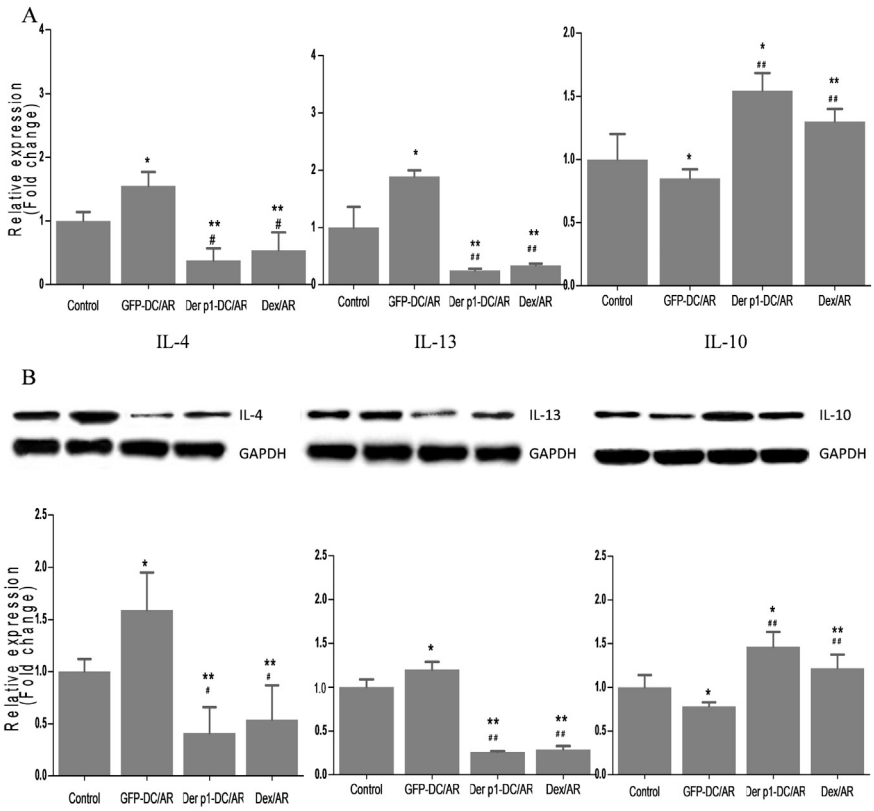

Fig. 5. Profiles of IL-4, IL-10, and IL-13 expression in the nasal mucosa. (A) IL-4, IL-10, and IL-13 gene expression in the nasal mucosa was detected using real-time polymerase chain reaction. GADPH was used as the housekeeping gene control. (B) Western blot analysis of IL-4, IL-10, and IL-13 proteins in the nasal mucosa of mice in four groups. GADPH was used as the housekeeping gene control. (C) Representative immunoblot of IL-4, IL-10, and IL-13 protein. The experiments had been repeated for > 3 times with consistent results. n = 6, mean ± SD. *: $p < 0.05$ vs. the control; #p: < 0.05 vs. the GFP-DC/AR group.

introduced as new immune therapy tools. Through transgene technology, target genes have been transfected into immune cells, which are then delivered to the human immune system to enhance antigen recognition and presentation. For example, using DCs targeting a Der p2 recombinant bacillus Calmette-Guérin, asthmatic mice exhibited a significant improvement in their airway [19]. Furthermore, directly introducing allergen transgenes into DCs, especially immature DCs of myeloid origin, can result in long-lasting multiple epitope antigens,

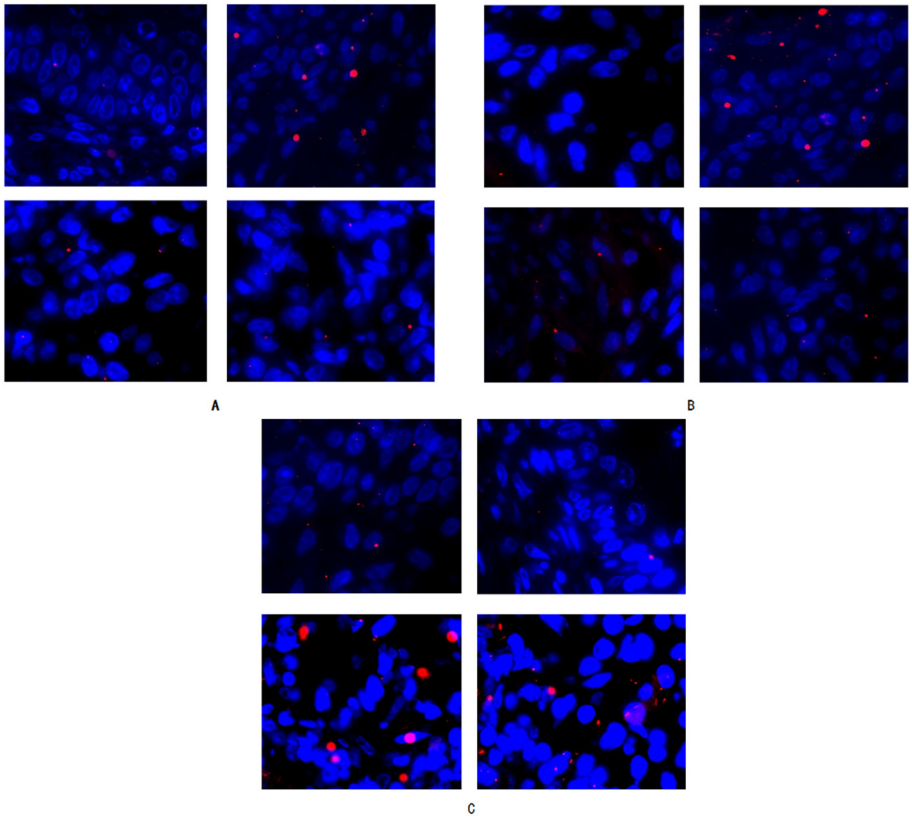

Fig. 6. Immunocytochemistry of IL-4, IL-10, and IL-13 in mice. Expression of IL-4 (A), IL-10 (B), and IL-13 (C) in the control (upper left), GFP-DC/AR (upper right), *Derp1*-DC/AR groups (lower left), and Dex/AR (lower right) was determined by immunocytochemistry. Immunoreactive IL-4 and IL-13, detected using a Cy3-labelled secondary antibody (red), were highly expressed in the nasal mucosa of mice in the GFP-DC/AR group and decreased in mice of the *Derp1*-DC/AR and Dex/AR groups, whereas they were barely expressed in the controls. IL-10 was expressed at a lower level in the nasal mucosa of mice in the GFP-DC/AR group compared to that of the control, and increased relative to the control in mice in the *Der p1*-DC/AR and Dex/AR groups. The experiments had been repeated for > 3 times with consistent results. Magnification × 1000 *$p < 0.05$ compared to control. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

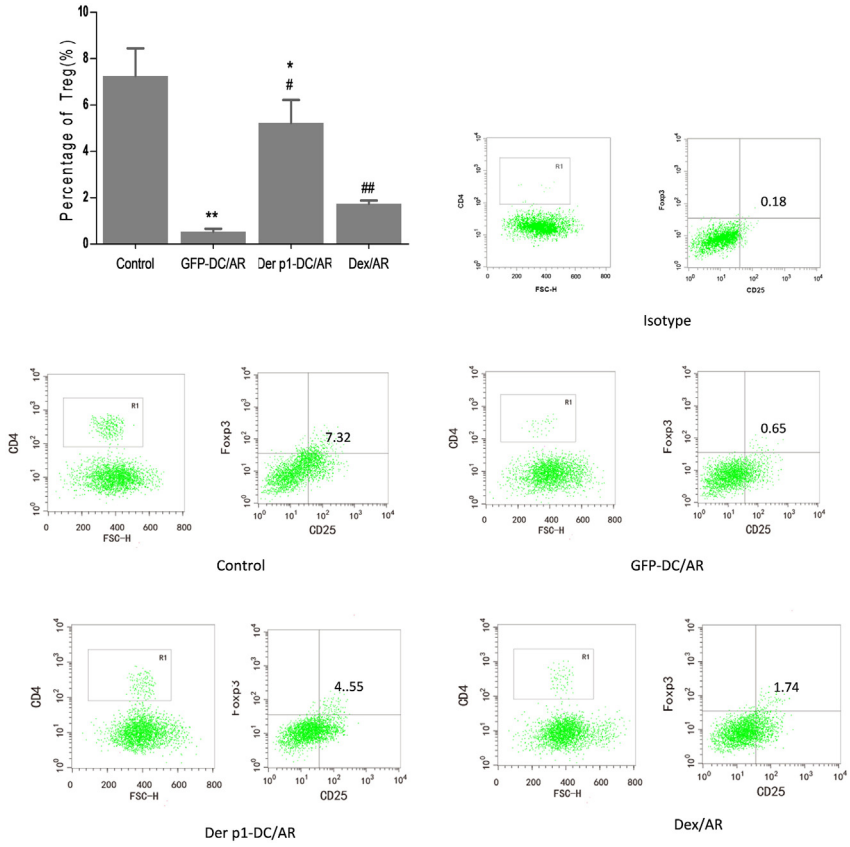

Fig. 7. Analysis of $CD4^+CD25^+Foxp3^+$ regulatory T (Treg) cells among peripheral blood mononuclear cells by flow cytometry. (A) Percentage of Treg cells in mice in the different groups. (B) Flow cytometric analysis of Treg cells. Peripheral blood mononuclear cells were isolated from fresh blood and stained with immunofluorescent antibodies against Treg surface markers (PE-conjugated anti-CD25, FITC-conjugated anti-CD4, and PE-Cy5-conjugated anti-Foxp3 antibodies). Dots in the right upper quadrants show individual cells that stained positive for Treg cells. The experiment has been repeated for > 3 times with consistent results. Each column and vertical bar represents the mean ± SD. *, **: Significantly different from the control ($p < 0.05$ and $p < 0.01$, respectively); #, ##: Significantly different from the AR group ($p < 0.05$ and $p < 0.01$, respectively).

enhanced DC uptake, DC processing, and allergen presentation that continuously and effectively induce T cell immune responses, thus desensitizing the body to the corresponding allergens [20].

Dust mites allergens are the most common domestic allergen, and Der p1 is the most common subtype of these allergens. In this present study, the *Der p1* gene was transfected into myeloid-derived immature DCs by a lentivirus, and high transfection efficiency was confirmed. In contrast to non-viral gene delivery systems, viral vector gene-transduced DCs elicit an immune response more efficiently than non-infected DCs [21]. Lentivirus is a retrovirus that can infect mitotic and non-mitotic cells [22]. Through lentiviruses, exogenous genes could be integrated into the host genome with high efficiency and be stably expressed, which is considered an advantage over other gene delivery systems. In our research, four maturation markers, CD11c, CD11b, CD59, and CD103, were highly expressed in Derp1-DC. During inflammation in response to allergen, DCs are recruited to the airway parenchyma. Inflammatory DCs are classically defined as $CD11b^+$, $CD11c^+$, and $CD59^+$ cells, and, in mice, these are the only DC subtype that express the high-affinity receptor for IgE [23,24]. Additionally, $CD103^+$ DCs were also found to prime Th2 responses to inhaled allergens [25]. Results of all of these studies indicate that *Derp1*-DC are mature and may prime divergent immune responses. Key co-stimulatory molecules CD80 and CD86 are found on DCs, activated B cells, and monocytes, and provide a costimulatory signal necessary for T cell activation [26]. The expression of surface markers in *Derp1*-DC suggests that lenti-*Derp1* can successfully promote DC maturation and differentiation, leading to a further immune response. It has been observed that *Derp1*-DC induced higher T cell proliferation than imDCs and it means *Derp1*-DC has immune responses in vivo.

In this study, compared to GFP-DC/AR mice, Der p1-DC/AR mice presented significantly decreased AR symptoms and lowered serum levels of histamine, IgE, and IgG1. In addition, *Derp1*-DC had the same therapeutic effect as dexamethasone treatment. Mouse IgG1 plays a major role in promoting inflammation, and its expression is regulated by Th2 cytokines. Therefore, histamine, IgE, and IgG1 in response to *Derp1*-DC can alleviate AR inflammation. This result is consistent with that of studies on immune tolerance to AR induced by *Derp1*-DC.

Treg cells play a key role in airway allergic disorders such as AR and asthma, and decreased numbers and function of Treg cells may result in airway allergic disease [27]. Clinical research has led to the same conclusion. Compared to the control group, the proportion of Treg cells is lower in the peripheral blood of patients with AR than in healthy controls [28]. Specific immunotherapy has been shown to improve AR symptoms by increasing the number of Treg cells [29]. Some research also demonstrated that glucocorticoids inhibit airway inflammation and hyperresponsiveness by increasing Treg numbers [30]. In this study, the proportion of Treg cells in peripheral mononuclear blood cells decreased after sensitization and increased after vaccine treatment, suggesting that *Derp1*-DC can increase the number of Treg cells, contributing to the amelioration of AR.

AR pathophysiology is identified as chronic airway inflammation, with infiltration of eosinophils, mast cells, and $CD4^+$ T lymphocytes that express Th2 cytokines such as IL-4, IL-5, and IL-13 [31,32]. IL-4 and IL-13 are immunoregulatory cytokines that are secreted predominantly by activated Th2 cells and serve as key mediators in the pathogenesis of allergic inflammatory diseases such as AR and asthma. IL-13 and IL-4 share the same receptor subunit and exert their biological function by binding the T-cell antigen receptor. IL-13 is secreted mainly by eosinophils and basophils. It then binds to IgE receptor with high affinity to initiate IgE-dependent inflammatory reactions and acts on B cells to produce IgE [33]. Therefore, a pathological increase in IL-4 and IL-13 levels is a critical mediator of AR inflammation. In this study, IL-4 and IL-13 mRNA levels were successfully downregulated in the nasal mucosa of mice treated with *Derp1*-DC compared to that of mice treated with immature DCs. Decreased IL-4 and IL-13 levels may inhibit the Th2 immune response, which in turn inhibits the synthesis and secretion of IgE, the maturation and function of inflammatory cells, and the synthesis of histamine, thereby reducing AR symptoms. These

results suggest that *Derp1*-DC can reduce inflammation, most likely by suppressing the expression of IL-4 and IL-13.

IL-10 is considered to have immunosuppressive functions and to modulate the activity of some key cell subsets involved in allergic reactions, including mast cells, Th2 cells, eosinophils, and DCs [34]. IL-10 also plays an important role in inducing immune tolerance in patients with allergic airway inflammation [35]. In this study, upregulation of IL-10 was observed in mice in the *Derp1*-DC/AR group, indicating that treatment with the modified DCs could induce immune tolerance in AR mice.

Conventional glucocorticoid therapy is a very effective treatment for allergic diseases. It can control inflammation well whether it is used systemically or locally. However, the long-term use of glucocorticoid can cause serious side effects. Even for topical use, there will also be some adverse reactions [36]. In this study, DC vaccine therapy can achieve the effects of glucocorticoid therapy, and it will become a very promising treatment without adverse side effects of glucocorticoid.

In conclusion, we successfully constructed *Der p1* gene-modified DCs. *Derp1*-DC were shown to induce immune tolerance in mice with AR through downregulation of IL-4 and IL-13, and upregulation of IL-10, with effects similar to those of dexamethasone treatment. This study indicates that this *Derp1*-modified DC vaccine has therapeutic potential against AR.

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Ethics approval and consent to participate

All animal care and experimental procedures were performed in accordance with the guidelines of the National Institutes of Health and were approved by the Tongji University Institution Animal Care and Use Committee (2013-DW-008).

Conflicts of interest

The authors declare no competing financial interest.

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