Article

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Dermatophagoides Pteronyssinus 1 Facilitates the Epithelial-Mesenchymal Transition of Nasal Mucosa Epithelial Cells to Impair Epithelial Barrier Function by Promoting NF-κB-Mediated Regulatory T Cells Differentiation and Interleukin-10 Secretion

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Background: Regulatory T cells (Treg) effectively impact allergic rhinitis (AR), the underpinning mechanism of which still warrants investigation. The predominant mite allergen, Dermatophagoides pteronyssinus 1 (Der p1), is the primary inducing factor for AR. Therefore, our study aims to explore whether Der p1 can induce AR by regulating Treg.

Methods: The AR mouse model was established by exposure to Dermatophagoides pteronyssinus 1 (Der p1). The behaviors and pathological alterations in the nasal mucosa tissues of mice were assessed, and biochemical indexes of mouse serum were examined. Determination concerning the functions of nasal mucosal epithelial barrier as well as the expressions of epithelial-mesenchymal transition (EMT)/inflammation-related factors was achieved using Western blot, fluorescein isothiocyanate-dextran (FD4) assay, and enzyme-linked immunosorbent assay (ELISA). Flow cytometry was used to determine the proportion of Treg in peripheral blood mononuclear cells (PBMCs). Furthermore, the conditioned medium of PBMCs treated with Der p1 (CM-Der p1) or nuclear factor-kappaB (NF-κB) inhibitors (CM-Der p1-NI) was used to culture nasal mucosal epithelial cells (NMECs), and then the vitality, barrier function, and EMT in NMECs were tested.

Results: Der p1 increased the frequency of rubbing and sneezing in mice, allergy-related biochemical indexes in serum, and interleukin (IL)-4, IL-6, and IL-10 levels in PBMCs ($p < 0.001$). Moreover, Der p1 increased the proportion of eosinophil infiltration in the nasal mucosa, impaired epithelial barrier function, EMT, NF-κB activation, and Treg differentiation ($p < 0.01$). However, these effects of Der p1 were reversed by NF-κB inhibitors ($p < 0.05$). Interestingly, NMECs cultured in CM-Der p1-NI showed higher viability, less IL-10 secretion, repaired barrier function, and inhibited EMT compared to the NMECs cultured in CM-Der p1 ($p < 0.05$).

Conclusions: Der p1 may stimulate IL-10 secretion through NF-κB pathway-mediated Treg differentiation, thereby inducing EMT in NMECs to impair epithelial barrier function.

Keywords: allergic rhinitis; regulatory T cells; Dermatophagoides pteronyssinus 1; interleukin-10; epithelial barrier function

Introduction

Allergic rhinitis (AR), a globally prevalent health-threatening disease, influences approximately 40% of adults and 25% of children around the world [1]. Common symptoms of AR include sneezing, nasal congestion, an itchy sensation in the nasal cavity, and a runny nose. Presently, AR is considered a type I hypersensitivity reaction mediated by Immunoglobulin E (IgE) [2]. Allergens enter the body through various routes such as the nasal mucosa, skin, and digestive tract, stimulating the production of IgE antibodies. IgE then binds to the surface receptors on mast cells and basophils, triggering a sensitized state in the body [2]. When the allergen re-enters the body, the specific IgE will be produced rapidly, and then combine with the above sensitized cells to induce their degranulation, releasing a series of active mediators to cause allergic symptoms, which can recruit a large number of inflammatory cells to infiltrate into the nasal mucosal tissue and produce more inflammatory factors to aggravate the nasal symptoms [2].

Over the past 30 years, allergen immunotherapy (AIT) by modulating the pathological mechanisms of allergy has evolved as the only therapeutic improvement of AR [3].

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

Notably, regulatory T cells (Treg) participate in AIT treatment, and can regulate the immune response through cell-to-cell contact or by cytokines production [4]. Furthermore, research has demonstrated that epithelial-mesenchymal transition (EMT) may contribute to the destruction and impairment of the nasal mucosal epithelial barrier [5]. Additionally, Treg may be involved in the cellular process of EMT, mediating the occurrence of diseases [6]. It is well known that interleukin (IL)-10, a crucial cytokine secreted by Treg, has been indicated to promote the EMT process in cancer cells [7]. Therefore, we speculated that Treg might regulate EMT within nasal mucosa tissue, affecting the progression of AR through the secretion of IL-10.

Alternatively, we investigated the activation of Treg in AR. The primary mite allergen, Dermatophagoides pteronyssinus 1 (Der p1), acts as the main inducing factor for AR [8]. Interestingly, the Der p1 protein shows cysteine protease activity [9]. Cysteine proteases such as MALT1 are implicated in maintaining Treg cell homeostasis by activating the nuclear factor-kappaB (NF-κB) pathway [10]. Despite advancements in understanding AR, the specific mechanisms linking Treg cells and EMT to its progression remain unclear. This study investigates a novel hypothesis that Der p1, through its cysteine protease activity, might activate Treg cells through the NF-κB pathway, thereby influencing EMT in nasal mucosa and potentially exacerbating AR.

Objects and Methods

Animals and Grouping

C57BL/6 mice (n = 24), weighing 10–12 g and aged 4 weeks, were obtained from the Hangzhou Medical College, Hangzhou, China. The mice were housed and bred in a pathogen-free environment. Moreover, the study design involving animal-related experiments was approved by the Ethics Committee of Zhejiang Baiyue Biotech Co., Ltd for Experimental Animals Welfare, China (Approval No. ZJBYLA-IACUC-20230220).

The AR mouse model was established following a previously described method [11]. During this process, the mice received intraperitoneal injections of Der p1-alum (10 μg or 100 μg Der p1 in 1 mg of aluminum hydroxide gel) on days 0, 7, and 14. Subsequently, they were exposed intranasally to Der p1 (20 μg), diluted in phosphate buffer saline (PBS, 40 mL, ms3560, Maokang, Shanghai, China), daily for 7 consecutive days, from day 21 to 27. Der p1 (HY-P75705) was obtained from MedChemExpress (Shanghai, China). Based on concentrations of Der p1-alum (0, 10, 100 μg), the mice were randomly divided into three groups: the Der p1 (0 μg) group, the Der p1 (10 μg) group, and the Der p1 (100 μg) group, with 8 mice per group. After the modeling procedure, one mouse was found dead in the Der p1 (10 μg) group and the Der p1 (100 μg) group. The overall success rate of the modeling procedure was 87.5%.

Evaluation of Nasal Symptoms

The number of rubbing and sneezing events within 20 minutes after the last exposure of mice to allergens was assessed by two impartial observers. On day 27, the mice were euthanized through cervical dislocation, and their mouse nasal mucosa tissues were collected. Furthermore, serum was obtained from the retro-orbital vessels of mice utilizing a syringe, while peripheral blood mononuclear cells (PBMCs) were isolated from the peripheral blood using a separation solution (P8620, Solarbio, Beijing, China).

Biochemical Measurements

The levels of IgE (HB-P9S923X) and Der p1-specific IgE/Immunoglobulin G (IgG) 1/IgG2a (HB-P9S2900X, HB-P9S3999X, HB-P9S3998X) in serum were evaluated using enzyme-linked immunosorbent assay (ELISA) kits (Huangbangbio, Guangzhou, China). Similarly, IL-4/6/10 (ZC-37986, ZC-37988, ZC-37962) levels in PBMCs were examined utilizing corresponding ELISA kits (Zcibio, Shanghai, China). The diluted sample or standard was transferred to the 96-well ELISA plates along with biotinylated antibodies. After incubation at 37 ℃ for 1 hour, the plates were washed using a plate washer (MultiWash, Molecular Devices, San Jose, CA, USA). After this, affinity streptomycin-HRP was added to each well, followed by incubation for 30 minutes. After washing the plates, substrates A and B were added to each well and incubated for 10 minutes. Subsequently, the reaction was stopped with the addition of a stop solution. Finally, the optical density (OD) value (450 nm) was immediately determined utilizing a microplate reader (FlexStation3, Molecular Devices, San Jose, CA, USA).

Histopathological Examination

The paraffin-embedded nasal mucosa tissue was cut into 4 μm thick sections. These tissue sections were sequentially immersed in gradient ethanol (100%–90%–80%–70%), distilled water, and xylene. Subsequently, tissue sections were treated with Hematoxylin and Eosin (H&E) solution (BA4025, Baso, Zhuhai, China) for color development and then observed using a microscope (NIB910, Boshida, Shenzhen, China).

Cellular Experiment Grouping

After euthanasia, the epithelial tissue from the nasal mucosa of normal mice was collected and immersed in 0.25% trypsin for 10 minutes. The supernatant was removed by centrifugation at 1000 r/min, and the resulting cell pellet nasal mucosal epithelial cells (NMECs) was resuspended in DMEM/F12 complete medium (SNM-004E, Sunncell, Wuhan, China).

Based on PBMCs derived from peripheral blood of normal mice, four groups were formed: the control group (normal culture), the Der p1 group (24-hour treatment with

5 µg/mL Der p1), the Der p1 + MG132 group, and the Der p1 + BAY117082 group (24-hour treatment with 5 µg/mL [12] Der p1 and 10 µM [13] MG132 or BAY117082). Both MG132 and BAY117082 served as NF-κB inhibitors. Subsequently, the conditioned medium of PBMCs from each of the above groups was collected for the culture of NMECs.

Flow Cytometry Analysis

Utilizing One Step Staining Mouse Treg Flow™ kit (136803, Biolegend, San Diego, CA, USA), the proportion of Treg cells in PBMCs was quantified through a flow cytometer (BriCyte E6, Mindray, Shenzhen, China) and analyzed using NovoExpress software (version 1.6.2, Agilent, Beijing, China).

Western Blot Analysis

Total protein was extracted from tissues or PBMCs using RIPA solution (BB3201, Beibokit, Shanghai, China) and quantified utilizing a BCA kit (BB-3401, Beibokit, Shanghai, China). The protein was resolved through SDS-PAGE (BB-3702, Beibokit, Shanghai, China) and subsequently transferred onto nitrocellulose membranes. The membranes were blocked with Western blocking buffer (BB-3512, Beibokit, Shanghai, China), followed by treatment with protein-specific antibodies. After this, the membranes were incubated overnight with primary at 4 °C. The following day, the membranes were probed with secondary antibodies. The immunoblots were developed using an ECL reagent (BB-3501, Beibokit, Shanghai, China) and examined through an imaging system (L00816C, Genscript, Piscataway, NJ, USA). Finally, the grayscale values of the protein bands were quantified using ImageJ software (1.8.0, National Institutes of Health, Bethesda, MD, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was utilized as an internal control. The list of antibodies used in Western blot is given in Table 1.

Measurement of Transepithelial Resistance (TER)

The cells were seeded into 24-well plates at a density of 5×10^4 cells per well and placed in Transwell inserts. The culture medium in the lower chamber was changed every 2 days. Furthermore, the TER of the cell monolayer was assessed using the epithelial voltohmmeter (EVOM)/EndOhm system (ENDOHM-24, WPI Inc, Sarasota, FL, USA).

Fluorescein Isothiocyanate-Dextran (FD4) Permeability Assay

The cells were seeded into 24-well plates at a density of 5×10^4 cells per well and placed in Transwell inserts. The upper chamber of the Transwell inserts was added with FD4 (MS0901, Maokangbio, Shanghai, China), followed by a 4-hour incubation. After this, the medium in the lower chamber was collected and examined using a fluorescence plate reader (PHERAstar®FSX, BMG LABTECH, Ortenberg, Germany), with the excitation wavelength of 493 nm and emission wavelength of 520 nm. Finally, signal intensity was analyzed using ImageJ software (1.8.0, National Institutes of Health, Bethesda, MD, USA), and the quantitative calculation formula was as follows: fluorescence intensity = (maximum intensity value – minimum intensity value)/total number of pixels.

Cell Viability

The cell viability was assessed employing the 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenytetrazoliumromide assay (MTT, BB-4201, Beibokit, Shanghai, China). The NMECs were seeded into 96-well plates at a density of 2000 cells per well, and subsequently treated with MTT reagent for 4 hours. The cells were washed with PBS, followed by a 10-minute incubation in a dissolved solution. Finally, the OD value at 490 nm was assessed using a microplate reader. The cell viability was determined as follows: Cell viability = [(treatment group OD value – blank well OD value)/(control well OD value – blank well OD value)] × 100%.

Statistical Analyses

Statistical analyses were performed using GraphPad Prism v8.0 (GraphPad Software, La Jolla, CA, USA). The data were expressed as mean ± standard deviation. The multi-group comparisons were conducted utilizing a one-way analysis of variance, followed by the post-hoc analysis of Dunnett test (Fig. 1,2) and Tukey test (Fig. 3,4). Statistical significance was considered at a p-value < 0.05.

Results

Der p1 Treatment Affected the Behavior, Nasal Mucosal Histopathology, and Epithelial Barrier Function in AR Mice

Following treatment with Der p1, mice experienced more rubbing and sneezing events (Fig. 1A, $p < 0.001$). Serum levels of total IgE and Der P1-specific IgE/IgG1/IgG2a were significantly increased in the 10 µg Der p1 (Der p1-10) and 100 µg Der p1 (Der p1-100) groups compared to those in the Der p1-0 group (Fig. 1B–E, $p < 0.001$). H&E staining revealed that both the Der p1-10 and Der p1-100 groups showed a higher proportion of eosinophils compared to the Der p1-0 group (Fig. 1F). Furthermore, we assessed the function of the epithelial barrier. Upon Der p1 treatment, a significant reduction in the expression of epithelial barrier function-related proteins (Occludin, Claudin-1, zonula occludens-1 (ZO-1), and junctional adhesion molecule-A (JAM-A)) (Fig. 1G–K, $p < 0.01$) was observed, along with the elevation in the fluorescence intensity of FD4 (Fig. 1L, $p < 0.001$). These findings indicated that the effects were more pronounced with a low dose of Der p1 than with a high dose of Der p1.

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

Table 1. A list of antibodies used in this study.

Name	Catalog	Molecular weight (kDa)	Dilution	Manufacturer
Occludin	#91131	65	1/1000	CST, Danvers, MA, USA
Claudin-1	ab180158	19	1/2000	Abcam, London, UK
ZO-1	ab276131	195	1/1000	Abcam, London, UK
JAM-A	ab270446	32	1/1000	Abcam, London, UK
N-cadherin	ab280375	99	1/1000	Abcam, London, UK
E-cadherin	ab231303	97	1/1000	Abcam, London, UK
Vimentin	ab8978	53	1/1000	Abcam, London, UK
IL-4	sc-53084	18	1/1000	SantaCruz, Santa Cruz, CA, USA
IL-6	ab290735	24	1/1000	Abcam, London, UK
IL-10	ab33471	20	1/1000	Abcam, London, UK
p-p65	ab76302	65	1/1000	Abcam, London, UK
t-p65	ab32536	65	1/1000	Abcam, London, UK
Foxp3	ab20034	50	1/1000	Abcam, London, UK
GAPDH	ab8245	36	1/10,000	Abcam, London, UK
Goat anti-rabbit	ab205718	—	1/2000	Abcam, London, UK
Goat anti-mouse	ab205719	—	1/2000	Abcam, London, UK

ZO-1, zonula occludens-1; JAM-A, junctional adhesion molecule-A; IL, interleukin; p-p65, phosphor-p65; t-p65, total p65; Foxp3, forkhead box protein 3; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

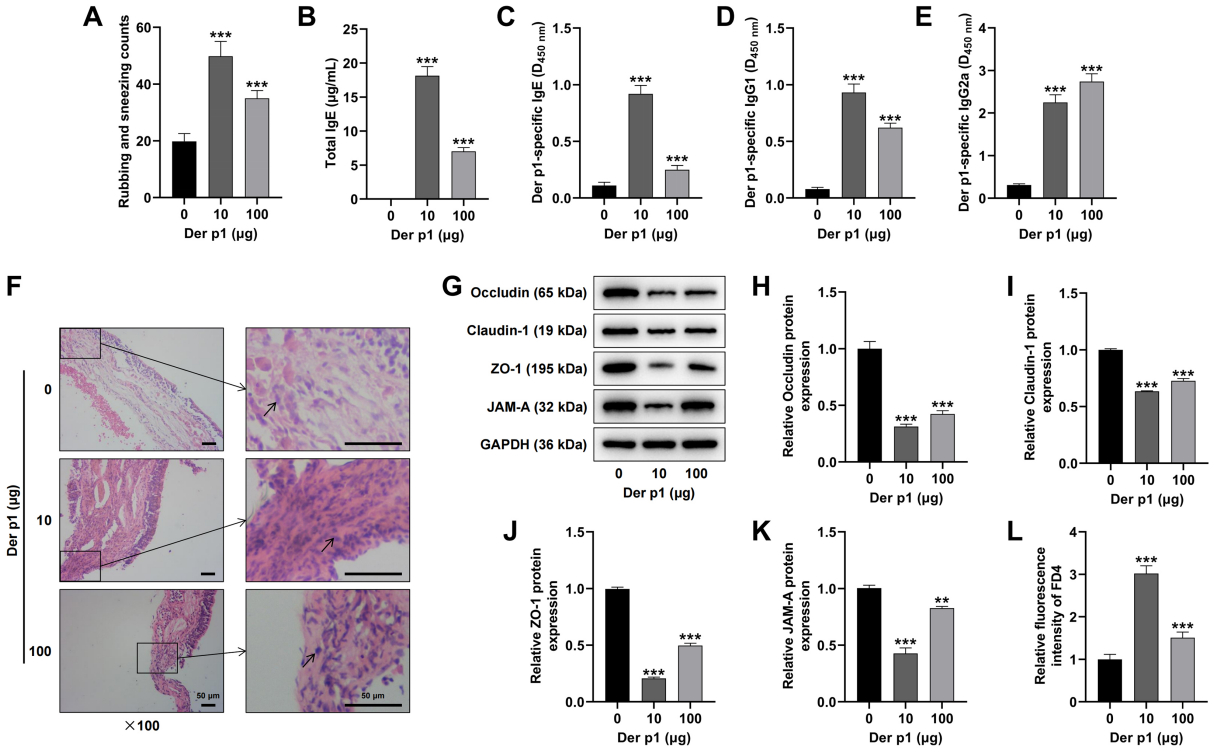

Fig. 1. The impact of Der p1 on the behavior, nasal mucosal histopathology, and epithelial barrier function of mice. The AR model was induced through exposure to Der p1-alum on days 0, 7, and 14 via intraperitoneal injection and subsequent intranasal treatment with Der p1 diluted in PBS daily for 7 consecutive days, from day 21 to 27. (A) Rubbing and sneezing counts. (B–E) Levels of total IgE and Der p1-specific IgE/IgG1/IgG2a in serum (enzyme-linked immunosorbent assay). (F) Images of nasal mucosa tissue (Hematoxylin and Eosin staining) (magnification: 100×; scale bar = 50 μm), and the arrows represent eosinophils. (G–K) Protein levels of epithelial barrier-related proteins (Western blot analysis, GAPDH as an internal control). (L) FD4 permeability assay in cells. $^{**}p < 0.01$, $^{***}p < 0.001$ vs. 0 group. Quantified values of three independently conducted experiments were presented as mean ± standard deviation. N = 3. AR, allergic rhinitis; FD4, fluorescein isothiocyanate-dextran; Der p1, Dermatophagoides pteronyssinus 1; IgE, Immunoglobulin E; PBS, phosphate buffer saline; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IgG, Immunoglobulin G.

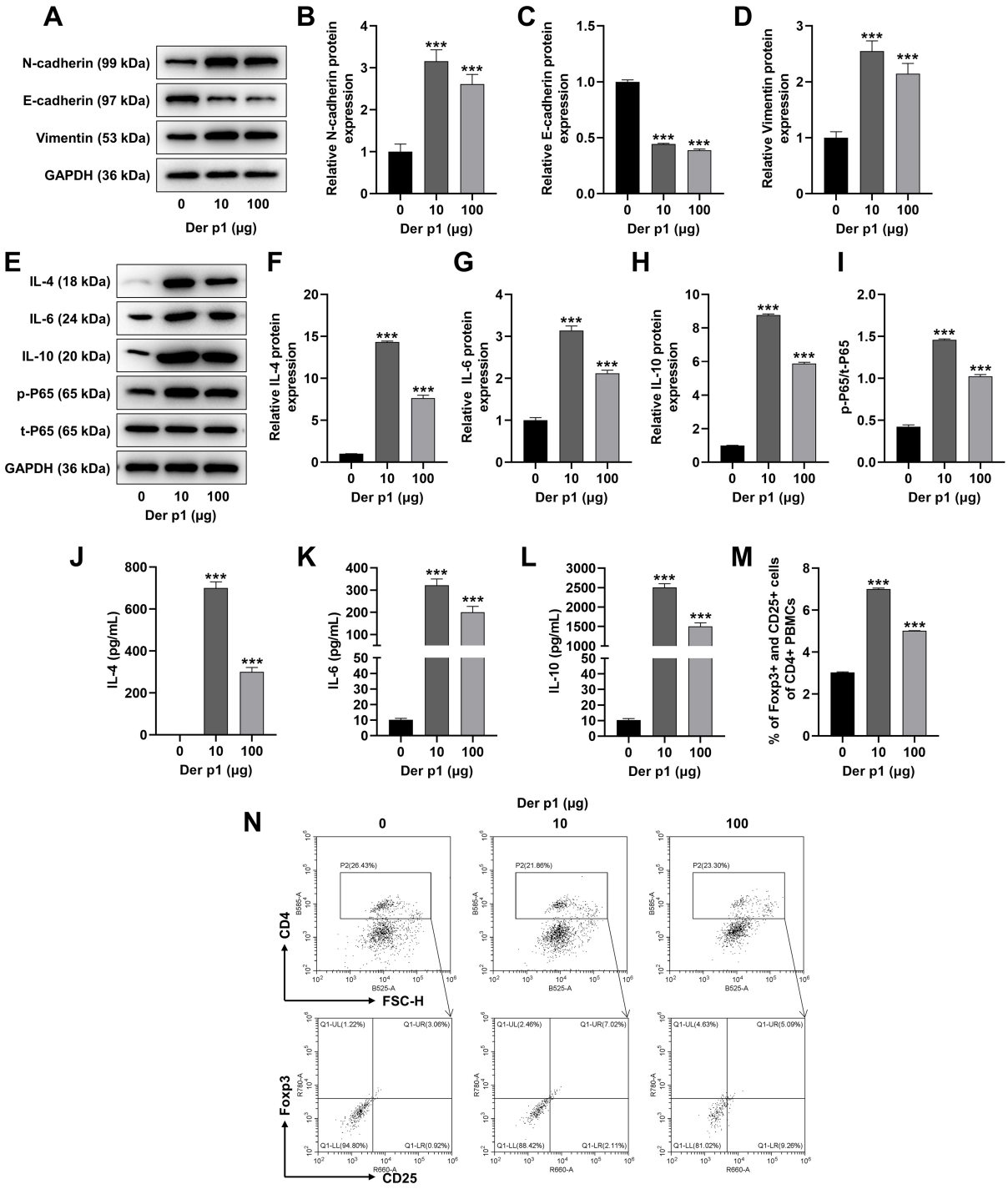

Fig. 2. Analyses on EMT-related proteins, inflammation-related cytokines, Treg cells, and NF-κB pathway in PBMCs of AR mice. The AR mouse model was induced by exposure to Der p1-alum on days 0, 7, and 14 through intraperitoneal injection and subsequent intranasal exposure to Der p1 diluted in PBS daily for 7 consecutive days from day 21 to 27. (A–D) Levels of EMT-related proteins and NF-κB pathway-related proteins (Western blot analysis, GAPDH as an internal control). (E–I) Levels of inflammation-related proteins (Western blot analysis, GAPDH as an internal control). (J–L) Levels of PBMCs-derived cytokines (enzyme-linked immunosorbent assay). (M,N) The proportion of Treg cells in PBMCs (flow cytometry). $^{***}p < 0.001$ vs. 0 group. Quantified values of three independently conducted experiments were presented as mean ± standard deviation. N = 3. NF-κB, nuclear factor-kappaB; AR, allergic rhinitis; EMT, epithelial-mesenchymal transition; PBS, phosphate buffer saline; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Treg, regulatory T cells; PBMCs, peripheral blood mononuclear cells; CD, cluster of differentiation; FSC-H, forward scatter-height.

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

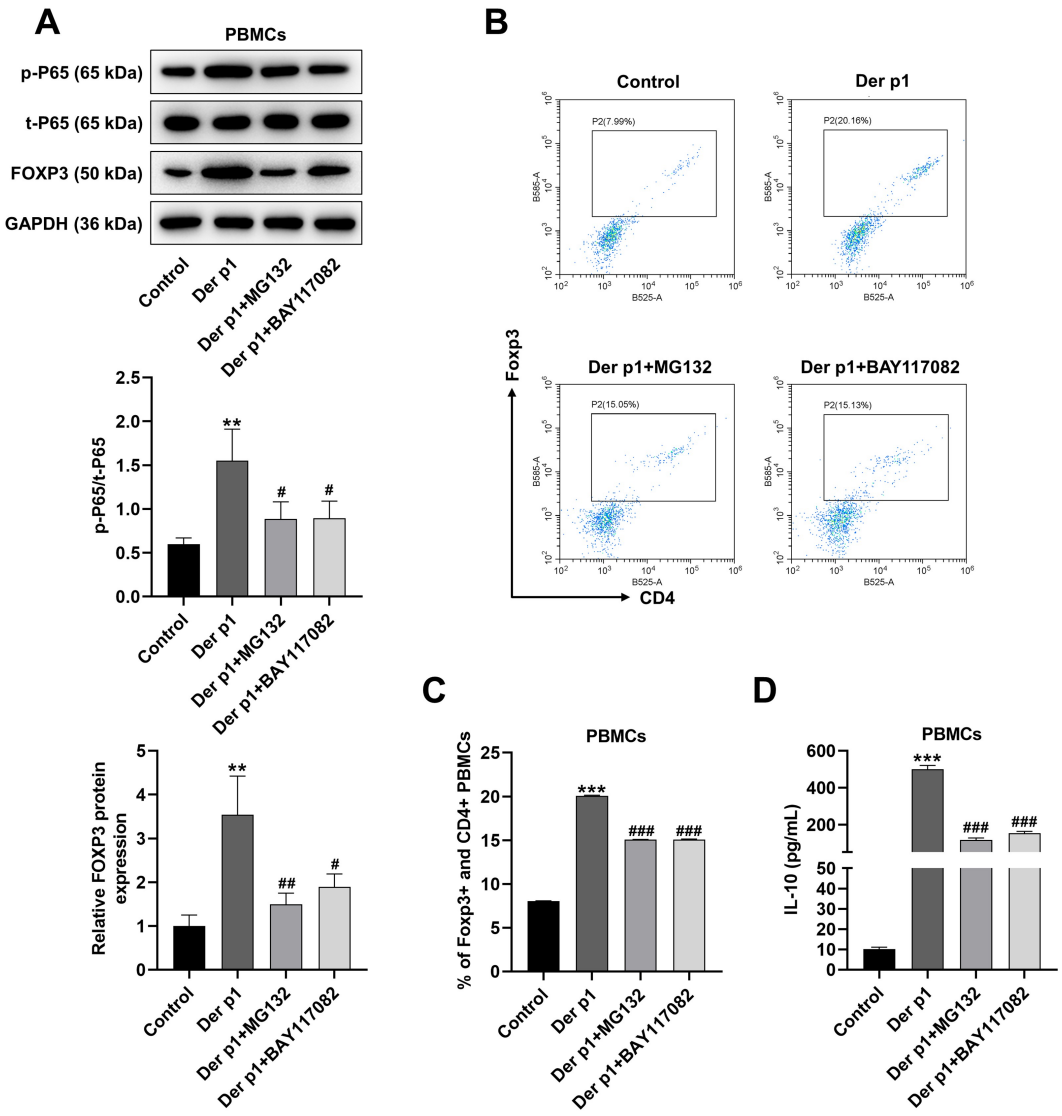

Fig. 3. NF-κB inhibitors reversed the effects of Der p1 on Treg cell proportion and IL-10 levels in PBMCs *in vitro*. PBMCs isolated from mice were co-treated with 5 μg/mL Der p1 and/or NF-κB inhibitors MG132 or BAY117082 for 24 hours. (A) Levels of NF-κB pathway-related proteins and FOXP3 in PBMCs (Western blot analysis, GAPDH as an internal control). (B,C) The proportion of Treg cells in PBMCs (flow cytometry). (D) IL-10 levels in cell medium (enzyme-linked immunosorbent assay). $^{**}p < 0.01$, $^{***}p < 0.001$ vs. the control group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. the Der p1 group. Quantified values of three independently conducted experiments were presented as mean ± standard deviation. N = 3. PBMCs, peripheral blood mononuclear cells; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Treg, regulatory T cells.

Der p1 Promoted EMT in Nasal Mucosa Tissue and Facilitated Treg Differentiation and NF-κB Pathway Activation in PBMCs

We investigated the effects of Der p1 treatment on the expression levels of proteins linked to inflammation and EMT. Western blot analysis showed that Der p1-10 and Der p1-100 diminished E-cadherin levels while increasing N-cadherin, Vimentin, the ratio of p-p65/t-p65, IL-4, IL-6, and IL-10 levels in nasal mucosa tissue (Fig. 2A–I, $p < 0.001$). Furthermore, ELISA results uncovered that Der p1-10 and Der p1-100 elevated IL-4, IL-6, and IL-10 levels in PBMCs (Fig. 2J–L, $p < 0.001$). Moreover, we observed that Der p1 increased the proportion of Treg in PBMCs (Fig. 2M,N, $p < 0.001$).

Der p1 Facilitated Treg Differentiation by Activating the NF-κB Pathway

To investigate whether Der p1 affected Treg differentiation by activating the NF-κB pathway, we performed *in vitro* experiments. Initially, an increase in p-p65/t-p65 levels was observed in PBMCs following Der p1 treatment, which was reversed by NF-κB inhibitor MG132 or

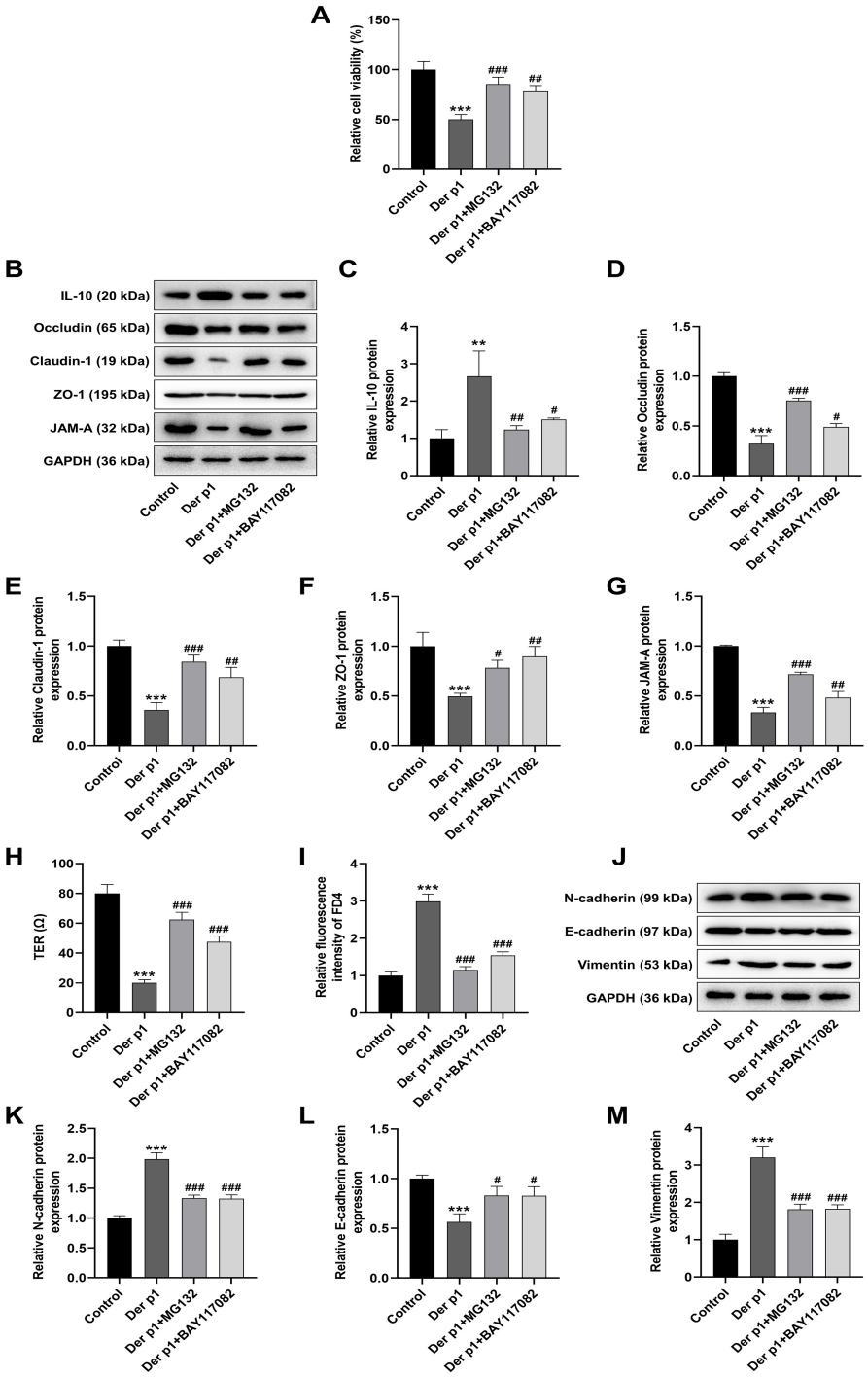

Fig. 4. Der p1 induced Treg differentiation through the NF-κB pathway to affect the barrier function of nasal mucosal epithelial cells. PBMCs isolated from mice were co-treated with 5 μg/mL Der p1 or NF-κB inhibitors MG132 or BAY117082 for 24 hours. Nasal mucosal epithelial cells isolated from mice were cultured in the conditioned medium of PBMCs. (A) The viability of nasal mucosal epithelial cells (3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenytetrazoliumromide assay). (B–G) Levels of IL-10 and epithelial barrier-related proteins (Western blot analysis, GAPDH as an internal control). (H) TER levels (EVOM/EndOhm system). (I) FD4 permeability assay in cells. (J–M) Levels of EMT-related proteins (Western blot analysis, GAPDH as an internal control). $^{**}p < 0.01$, $^{***}p < 0.001$ vs. the control group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. the Der p1 group. Quantified values of three independently conducted experiments were presented as mean ± standard deviation. N = 3. TER, transepithelial resistance; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; FD4, fluorescein isothiocyanate-dextran; EMT, epithelial-mesenchymal transition; EVOM, epithelial voltohmmeter.

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

BAY117082 (Fig. 3A, $p < 0.05$). Furthermore, the upregulation of forkhead box protein 3 (Foxp3) (Fig. 3A, $p < 0.01$), the increased proportion of Treg (Fig. 3B,C, $p < 0.001$), and the upregulation of IL-10 (Fig. 3D, $p < 0.001$) in Der p1 group were all negated by NF-κB inhibitors (Fig. 3A–D, $p < 0.05$).

Der p1 Affected the Function of NMECs through NF-κB Pathway-Mediated Treg Differentiation

To investigate whether Der p1 affected the function of NMECs by mediating Treg differentiation, we used the conditioned medium of PBMCs in each group to culture NMECs. Under the culture of the conditioned medium derived from Der p1-treated PBMCs, NMECs exhibited significantly reduced viability (Fig. 4A, $p < 0.001$) and TER (Fig. 4H, $p < 0.001$), elevated IL-10, N-cadherin, and Vimentin levels (Fig. 4B,C,J,K,M, $p < 0.01$), alleviated Occludin, Claudin-1, ZO-1, JAM-A, and E-cadherin levels (Fig. 4D–G,L, $p < 0.001$) and higher fluorescence intensity of FD4 (Fig. 4I, $p < 0.001$). However, these effects were all reversed by NF-κB inhibitors (Fig. 4A–M, $p < 0.05$).

Discussion

House dust mite (HDM) is the primary allergen responsible for perennial AR, with Der p1 recognized as its main allergenic protein [14]. In this study, we initially focused on AR mice induced by Der p1 as the research subject. We observed that Der p1 induced an obvious nasal inflammatory response and increased secretion of Th2 cytokines (IL-4 and IL-6) in nasal mucosa tissues and PBMCs. These cytokines mediate B cell differentiation into plasma cells and induce the production of IgG1, IgE, and IgG2a [15]. This finding is consistent with our results indicating that Der p1 elevated the levels of total IgE and Der p1-specific IgE/IgG1/IgG2a.

Additionally, Th2 cytokines can regulate AR by downregulation of tight junction (TJ) in epithelial cells. In AR, the nasal epithelial barrier is the first line of defense against allergen invasion, and the destruction of epithelial barrier function leads to increased permeability, facilitating allergen penetration [16]. The junctions between epithelial cells are associated with the apical junction complex (AJCs), with TJ being the most robust epithelial junction within AJCs, which consists of occludin, claudin, and JAMs [16]. Previous studies have highlighted that IL-4 can prevent ZO-1 and Occludin expressions [17], and anti-IL-4 therapy can mitigate epithelial barrier dysfunction in patients with AR [18]. IL-6 is highly expressed in AR patients, with its concentration increasing with disease progression [19]. IL-6 is a vital mediator of eosinophilic inflammation, which is crucial in producing nasal secretions [20]. Consistently, Der p1 in our study simultaneously upregulated IL-6 level and promoted eosinophil infiltration in nasal mucosal tissues. Furthermore, Wan *et al.* [21] reported that Der p1 promoted the trans-epithelial delivery of allergens by disrupting TJ. In our study, Der p1 down-regulated the epidemic barrier function-related proteins (Occludin, Claudin-1, ZO-1, and JAM-A), increasing epithelial permeability.

The destruction of epithelial barrier function is associated with the aggravation of EMT. A study has indicated a significant increase in the expression of EMT-related proteins in patients with chronic rhinosinusitis (CRS) [22]. EMT can result in the loss of epithelial characteristics, leading to epithelial leakage and facilitating allergens invasion into the body [23]. Consistently, we observed abnormal EMT-related protein expression in the nasal mucosa of Der p1-induced mice. Notably, a study by Song *et al.* [24] indicated that miR-192-5p/RB1 induced Treg cell differentiation by activating NF-κB axis-mediated IL-10 secretion, thereby aggravating the EMT in gastric cancer cells . Therefore, the increase in IL-10 secretion mediated by the NF-κB axis could be the underlying cause of EMT aggravation. Herein, we found that Der p1 promoted NF-κB activation, Treg differentiation, and IL-10 secretion, indicating that the Der p1-mediated NF-κB axis might affect EMT through IL-10 in AR. IL-10, a regulatory cytokine secreted by Treg, shows a significant increase in expression following Der p1 stimulation [25]. Previous findings have indicated the anti-inflammatory impacts of IL-10. However, clinical experimental results also show that high-doses of IL-10 can exert pro-inflammatory effects on patients with inflammatory diseases [26]. Therefore, the role of IL-10 remains controversial. While no study has reported the regulatory effect of IL-10 on EMT in NMECs, its potential to induce EMT has been recognized [27]. Additionally, we found that the up-regulation of IL-10 induced by Der p1 was reversed by inhibitors of NF-κB. A previous study indicated that NF-κB p65 combined with the IL-10 promoter enhanced the expression of IL-10 [28]. Therefore, the Der p1-mediated up-regulation of IL-10 in AR may rely on activation of NF-κB. Moreover, study has highlighted that enhanced NF-κB signaling can directly promote Foxp3 (a vital transcription factor for Treg) transcription, indicating the significance of NF-κB activation in the development of Treg [29], which further explains why the NF-κB inhibitor in our study reversed the differentiation of Treg induced by Der p1. Subsequently, we used a conditioned medium derived from Der p1 and/or NF-κB inhibitor-treated PBMCs to culture NMECs. We found alterations in the viability, TJs, permeability, and EMT of NMECs, suggesting that NF-κB-mediated Treg differentiation was the underlying cause of these changes in the characteristics of NMECs. Furthermore, considering that IL-10 is mainly secreted by Treg and its production can further enhance the differentiation of Treg [30], the positive feedback pathway of IL-10-Treg-IL-10, under the activation of NF-κB, may continuously affect the epithelial barrier function of NMECs by

promoting EMT. However, this study did not delve into the mechanisms related to Treg cell differentiation using Treg cells, which will be addressed in the future.

Conclusions

In summary, we elucidate that Der p1 promotes Treg differentiation and IL-10 secretion by activating the NF-κB pathway, potentially affecting the epithelial barrier function by promoting EMT in NMECs. This study holds promise to contribute to the immunotherapy of AR and offers new insights into the multiple roles of IL-10 in AR.

Availability of Data and Materials

All experimental data included in this study can be obtained by contacting the corresponding author if needed.

Author Contributions

LL, WL designed the research study. LL and WF performed the research. LL and JW provided help and advice on the experiments. SZ analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study design involving animal-related experiments was approved by the Ethics Committee of Zhejiang Baiyue Biotech Co., Ltd for Experimental Animals Welfare, China (Approval No. ZJBYLA-IACUC-20230220).

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Conflict of Interest

The authors declare no conflict of interest.

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