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Interleukin 1β Mediates the Pathogenesis of Nasal Mucosal Epithelial Barrier Dysfunction in Allergic Rhinitis

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Background: The nasal mucosal epithelial barrier is the primary site of allergic rhinitis (AR). Interleukin-1β (IL-1β), as a crucial factor in immune inflammation, not only plays a crucial role in hypersensitivity reactions but also affects the digestive mucosa and skin epithelial barrier. However, the role of IL-1β in the nasal mucosal epithelial barrier in AR has not been reported, and this study aimed to investigate the effect and possible mechanisms involved.

Methods: Dermatophagoides pteronyssinus 1 was used as an allergen to construct an AR mouse model and stimulate human nasal mucosal epithelial cells (HNEpCs) and observe the expression changes of IL-1β and epithelial barrier indicators CLDN1 and OCLN in mouse nasal mucosa and HNEpCs. Then, the possible mechanisms of action were explored via exogenous IL-1β stimulation and pharmacological inhibition of IL-1β or its receptor interleukin-1 receptor type 1 (IL-1R1).

Results: The results showed that Dermatophagoides pteronyssinus 1-primed mouse nasal mucosa or human HENpCs had increased expression of IL-1β and decreased CLDN1 and OCLN, and IL-1β could directly lead to reduced expression of epithelial barrier indexes in HNEpCs. In addition, inhibition of IL-1β or IL-1R1 can effectively alleviate the damage to the epithelial barrier.

Conclusion: IL-1β has a destructive effect on the nasal mucosal epithelial barrier in AR, and inhibition of IL-1β or its receptor IL-1R1 can effectively protect the nasal mucosal barrier. IL-1β is a potential target for the treatment of AR.

Keywords: interleukin-1β, allergic rhinitis, nasal mucous membrane, epithelial barrier, DerP1

Introduction

Allergic rhinitis (AR) is a localized chronic, non-infectious inflammation of the nasal mucosa induced by contact with individual-specific allergens. Its symptoms seriously affect people's normal lives and work-study efficiency.[1] The epithelial cells of the normal nasal mucosa, which are in close contact with the external environment, act as a physiological barrier to prevent pathogenic bacteria from invading the area. When the barrier is damaged, allergens penetrate deeper into the inter-epithelial and subepithelial regions of the mucosa. This stimulates allergy-related effector cells or factors, which will promote the development of type I hypersensitivity reactions.[2,3] Furthermore, the increase of those allergy-related factors also contributes to the damage of the epithelial barrier, ultimately leading to the formation of a vicious circle of "physical barrier destruction-immune imbalance" in the allergen-stimulated nasal mucosa, which is a key link that is frequently disregarded.[4]

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Research on the structure and function of the mucosal barrier has advanced owing to improvements in etch electron microscopy, transmission electron microscopy, and other biotechnological techniques. Several studies have confirmed that the dynamics of the nasal mucosal epithelial barrier are implicated in almost the entire pathophysiological process of the nasal immune response in AR.[5,6] The intact epithelial barrier defends against allergen invasion by allergens that seep in owing to the structural and functional impairment of the nasal barrier and finally to the immune-inflammatory response of the body rupturing the tight junctions (TJs) between epithelial cells.[7,8] Although the "epithelial barrier hypothesis" has been mentioned frequently during the investigation of AR, its specific mechanism of action has not yet been elucidated, and the current studies are still in the exploratory stage.[9] Therefore, identifying its key targets of action, reducing damage to the epithelial barrier, and preserving or regaining its structural integrity and function in the prevention and treatment of AR can prevent disease occurrence in the early stage and reduce the use of therapeutic drugs in the later stages of AR.

As an important inflammatory factor in the body's immune response, interleukin-1β (IL-1β) can be produced by various nucleated cells, including nasal mucosal epithelial cells.[10,11] IL-1β, as a downstream effector inflammatory factor after inflammasome activation, has been previously shown in a large number of previous studies to be significantly overexpressed in allergic diseases and to be closely associated with disease severity.[12–14] In addition, it has also been suggested that IL-1β may be a potential biomarker for AR.[15] Inflammasome has a crucial role in the innate immunity of the body, and it can mediate the disease progression by increasing the inflammatory response and affecting antigen presentation in allergic airway diseases, including AR.[16–18] Meanwhile, IL-1β can also participate in Th1/Th2 immune imbalance via different pathways, promotes eosinophil activation, and plays an essential role in upper airway tissue remodeling.[19] Thus, effective inhibition of IL-1β or its receptor can reduce the local inflammation of AR to a certain extent and control the progression of the disease.[20,21] Furthermore, it has been previously shown that IL-1β has different coregulatory effects on the epithelial barrier in the gastrointestinal tract, lungs, skin, etc.[22,23] However, how it is specifically implicated in the disease process as a key immunological factor in AR remains unknown.

Consequently, we looked into how IL-1β functions and regulates the pathophysiology of AR by affecting the nasal mucosal epithelial cell barrier, which in turn facilitates the formation of AR. We established animal and cellular models of AR to investigate its effects and potential mechanisms of action. To clarify the nosogenesis of AR as well as improving its clinical diagnosis and therapy, we provide new ideas and possible intervention targets.

Materials and Methods

Animals

The animal research protocol was approved by the Ethics Committee of Yantai Yuhuangding Hospital (2022–104). C57BL/6 male mice, aged 6–8 weeks and weighing 22–25 g, were procured from Jinan Pengyue Laboratory Animal Breeding Co. Ltd. (Shandong, China). All mice were housed in a pathogen-free animal laboratory. The animals used in this research were handled according to "the Guide for the Care and Use of Laboratory Animals (ISBN: 9787547812969)".

Dermatophagoides Pteronyssinus 1 (DerP1)-Induced Mice Model of AR and Behavioral Tests

In this study, house dust mite was used as an allergen for in vivo and in vitro experiments. The house dust mite is the most common allergen in daily life, and its main protein component, DerP1 (XPB91D3A2.5, Greer, USA), which is used in Steelant B's approach,[24] was used for building the AR model. The mice were randomly divided into two groups: AR and sham. On day 1, the mice in the AR group were intranasally stimulated bilaterally with 1 μg of DerP1 dissolved in 50 μL of 0.9% NaCl. From days 8 to 12, the mice were continually stimulated bilaterally with 10 μg of DerP1 dissolved in 50 μL of 0.9% NaCl per day. Moreover, 0.9% NaCl was used to intranasally excite the sham group.

After the modeling was completed, the frequency of nose scratches in a single mouse over the course of 5 min was videotaped to assess the mouse's allergic response. Mouse serum and nasal mucosal tissues were collected for subsequent experiments.

Culture and Treatment of Human Nasal Epithelial Cells (HNEpCs) and T Cells

The HNEpCs were purchased from the BeNa Co., Ltd. (Beijing, China). The cells were grown at 37°C and 5% CO2 in MEM medium (C3050, BioInd, Israel) supplemented with 10% fetal bovine serum (C04001, BioInd, Israel) and 1% Penicillin-Streptomycin Solution (C0222, Beyotime, Beijing, China). After the cell density reached 70–80%, cells were cultured in medium with 40 μg/mL DerP1 or 10 pg/mL IL-1β (10139-HNAE, Sino, Beijing, China) for 24 h. If cells needed recovery trials, cells had to wait until they attained a density of 60–70%, and DerP1 was added for 24 h of culture after the addition of Diacerein (50 μM; HY-N0283, MCE, Shanghai, China), AF12198 (10 nM; HY-P1110, MCE, Shanghai, China) or Belnacasan (50 μM; HY-13205, MCE, Shanghai, China) intervention two hours beforehand.

The CD4+ T Cell were purchased from the Jinyuan Biotechnology Co., Ltd. (Shanghai, China). The cells were grown in RPMI 1640 medium (CGM112.05, Cellmax, Beijing, China) supplemented with 10% fetal bovine serum and 1% Penicillin-Streptomycin Solution. The cells are activated by incubating with human CD3/CD28 T cell activator (10971, Stemcell, Canada) during the cultivation process. The cell were cultured in medium with 10 pg/mL IL-1β for 24 h, and the inhibitor intervention group will add AF12198 (10 nM) two hours prior to stimulation.

Enzyme-Linked Immunosorbent Assay (ELISA)

The ELISA kits for mouse immunoglobulin E (Ig E; EMC117.96), IL-4 (EMC003.96), IL-5 (EMC108.96), and human IL-1β (EHC002b.96) used in the research were purchased from Neobioscience (Shenzhen, China). The ELISA kits for human IL-4 (RK00003), IL-5 (RK00033), and IL-13 (RK00034) were purchased from ABclonal Biotechnology Co., Ltd. (USA). The protein levels of Ig E, IL-4, and IL-5 in mouse serum, as well as the protein concentration of IL-1β in DerP1-treated or control cell culture medium and the protein concentrations of IL-4, IL-5, IL-13 in CD4+ T cell culture medium treated and blocked with IL1β, were determined using ELISA in strict accordance with the instructions for use. A Multiskan FC zymograph (Thermo Fisher Scientific, USA) was used to measure absorbance at 450 nm to calculate protein concentration.

Histopathological Staining

The entire mouse nose was collected, fixed in 4% paraformaldehyde (Biosharp, Anhui, China), decalcified, and embedded in paraffin (Leica, Wetzlar, Germany). The tissues were sliced at a thickness of 4 μm, and the mouse nasal structures were stained with kits of hematoxylin and eosin staining (C0105, Beyotime, Beijing, China) and Periodic Acid-Schiff staining (C0142M, Beyotime, Beijing, China). The specific steps of the experiments were referred to the instructions carefully. After staining was completed, the tissue was observed under a microscope (DMLB2, Leica, Germany) for comparison.

Immunofluorescence (IF)

Using the above method, paraffin sections of mouse nasal mucosa were sliced at a thickness of 4 μm and subjected to hydration and antigen repair, or the HNEPC crawls were fixed with 4% paraformaldehyde. Subsequently, they were blocked with 5% bovine serum albumin solution (phosphate-buffered saline) for 1 h at room temperature. The samples were incubated with CLDN-1 antibody (1:400; 13050-1-AP, Proteintech, Wuhan, China) or OCLN antibody (1:400; AF7644, Beyotime, Beijing, China) at 4°C overnight. The next day, the tissue slices or cells were washed three times with phosphate-buffered saline and incubated with YF®488 Goat Anti-Rabbit IgG (1:500; Y6105; UELandy, Jiangsu, China) for two h at room temperature. The slices were washed again, incubated with DAPI (C1005, Beyotime, Beijing, China) for 10 min, washed, and sealed with an antifade mounting medium (S2130, Solarbio, Beijing, China). The stained samples were observed under an Axio Observer 7 microscope (Carl Zeiss AG, Germany).

Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR)

Total RNA was extracted from mouse nasal mucosa tissue or HNEpCs cells using VeZol reagent (R411-01, Vazyme, Jiangsu, China). After measuring the RNA concentration, it was reverse transcribed to cDNA using the HiScript II Q RT SuperMix for qPCR (+gDNA wiper) kit (R223-01; Vazyme, Jiangsu, China). Then, qPCR was performed using the

ChamQ SYBR qPCR Master Mix kit (Q321-02, Vazyme, Jiangsu, China), and the steps were performed on the FTC-3000P system (Funglyn Biotech, Canada), with the program set up as follows: ① 95°C for 5 min; ② 40 cycles: 95°C for 10 s, 60°C for 30 s; ③ 95°C for 1 min, 60°C for 1 min, 95°C for 0.5 s. The data obtained were analyzed using the ΔΔCt method, which uses glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal control to calculate the relative RNA expression levels. The primers (Sangon Biotech, Shanghai, China) used for qPCR are listed below: mouse *Gapdh*: forward: 5′-AGGTCGGTGTGAACGGATTTG-3′; reverse: 5′-TGTAGACCATGTAGTTGAGGTCA-3′; Mouse *Ocln*: forward: 5′-TTGAAAGTCCACCTCCTTACAGA-3′; reverse: 5′-CCGGATAAAAAGAGTACGCTGG-3′; Mouse *Cldn1*: forward: 5′-GGGGACAACATCGTGACCG-3′; reverse: 5′-AGGAGTCGAAGACTTTGCACT-3′; Mouse *Il-1b*: forward: 5′-GCAACTGTTCCTGAACTCAACT-3′; reverse: 5′-ATCTTTTGGGGTCCGTCAACT-3′; Mouse *Il-1rn*: forward: 5′-TGTGCCTGTCTTGTGCCAAGTC-3′; reverse: 5′-GCCTTTCTCAGAGCGGATGAAG-3′; Human *GAPDH*: forward: 5′-GCACCGTCAAGGCTGAGAAC-3′; reverse: 5′-TGGTGAAGACGCCAGTGGA-3′; Human *OCLN*: forward: 5′-ATGGCAAAGTGAATGACAAGCGG-3′; reverse: 5′-CTGTAACGAGGCTGCCTGAAGT-3′; Human *CLDN1*: forward: 5′-GTCTTTGACTCCTTGCTGAATCTG-3′; reverse: 5′-CACCTCATCGTCTTCCAAGCAC-3′; Human *IL-1B*: forward: 5′-AGCTACGAATCTCCGACCAC-3′; reverse: 5′-CGTTATCCCATGTGTCGAAGAA-3′; Human *IL-1RN*: forward: 5′-ATGGAGGGAAGATGTGCCTGTC-3′; reverse: 5′-GTCCTGCTTTCTGTTCTCGCTC-3′; Human *CASP1*: forward: 5′-GCTGAGGTTGACATCACAGGCA-3′; reverse: 5′-TGCTGTCAGAGGTCTTGTGCTC-3′; Human *IL-18*: forward: 5′-GATAGCCAGCCTAGAGGTATGG-3′; reverse: 5′-CCTTGATGTTATCAGGAGGATTCA-3′.

Western Blot Analysis (WB)

Mouse nasal mucosal tissues or HNEpCs were lysed with radioimmunoprecipitation assay buffer (R0020, Solarbio, Beijing, China) containing phenylmethanesulfonyl fluoride (EA0005, Sparkjade, Shandong, China), and protein concentration was determined using a BCA kit (EC0001, Sparkjade, Shandong, China). Protein loading buffer (P1040, Solarbio, Beijing, China) was added to an equal amount of protein sample in a 1:4 ratio. The obtained mixture was electrophoresed on a 10% or 12% sodium dodecyl sulfate-polyacrylamide gel, and the separated proteins were transferred to a nitrocellulose membrane. Subsequently, the membranes were incubated with a 5% bovine serum albumin-blocking solution at room temperature for 2 h. The primary antibodies (CLDN1, 13050-1-AP, Proteintech, Wuhan, China; OCLN, AF7644, Beyotime, Beijing, China; IL-1β, ab254360, Abcam, UK; Interleukin-1 receptor antagonist (IL-1RA) AF7218, Beyotime, Beijing, China; tubulin, 10068-1-AP, Proteintech, Wuhan, China; GAPDH, AF1186, Beyotime, Beijing, China; CASP1, 22915-1-AP, Proteintech, Wuhan, China; IL-18, 10663-1-AP, Proteintech, Wuhan, China) were diluted at a ratio of 1:1000, and the membrane was submerged at 4°C overnight. On the second day, the membranes were washed three times with tris buffered saline solution containing 0.1% Tween-20 (ST825, Beyotime, Beijing, China) and then incubated with horseradish peroxidase-conjugated secondary antibodies [Goat Anti-Rabbit IgG (H6162) or Goat Anti-Mouse IgG (H6161), UELandy, Jiangsu, China], whose strips corresponded to the primary antibody species, for 2 h at room temperature. Then, the membranes were washed three times with tris buffered saline with Tween-20 again. Finally, an enhanced chemiluminescence kit (ED0015, Sparkjade, Shandong, China) was used for chemiluminescence detection on a ChemiScope6200 Touch (Clinx, Shanghai, China), and the relative intensities of the protein bands were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Statistical Analysis

The data of this study are expressed as mean ± standard error of the mean (SEM), and GraphPad Prism 9 software (San Diego, CA, USA) was used to analyze the experimental data statistically, in which a *t*-test was used for the comparison between two groups, and one-way ANOVA was used for the comparison among multiple groups. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ all indicate that the difference is statistically significant.

Results

AR Mice Exhibit Damage to the Nasal Mucosal Epithelial Barrier

After induction of the AR mice model with DerP1 (Figure 1A), the AR group mice exhibited increased nasal scratching as well as significantly higher levels of serum Ig E, IL-4, and IL-5 than the sham group mice (Figure 1B–E).

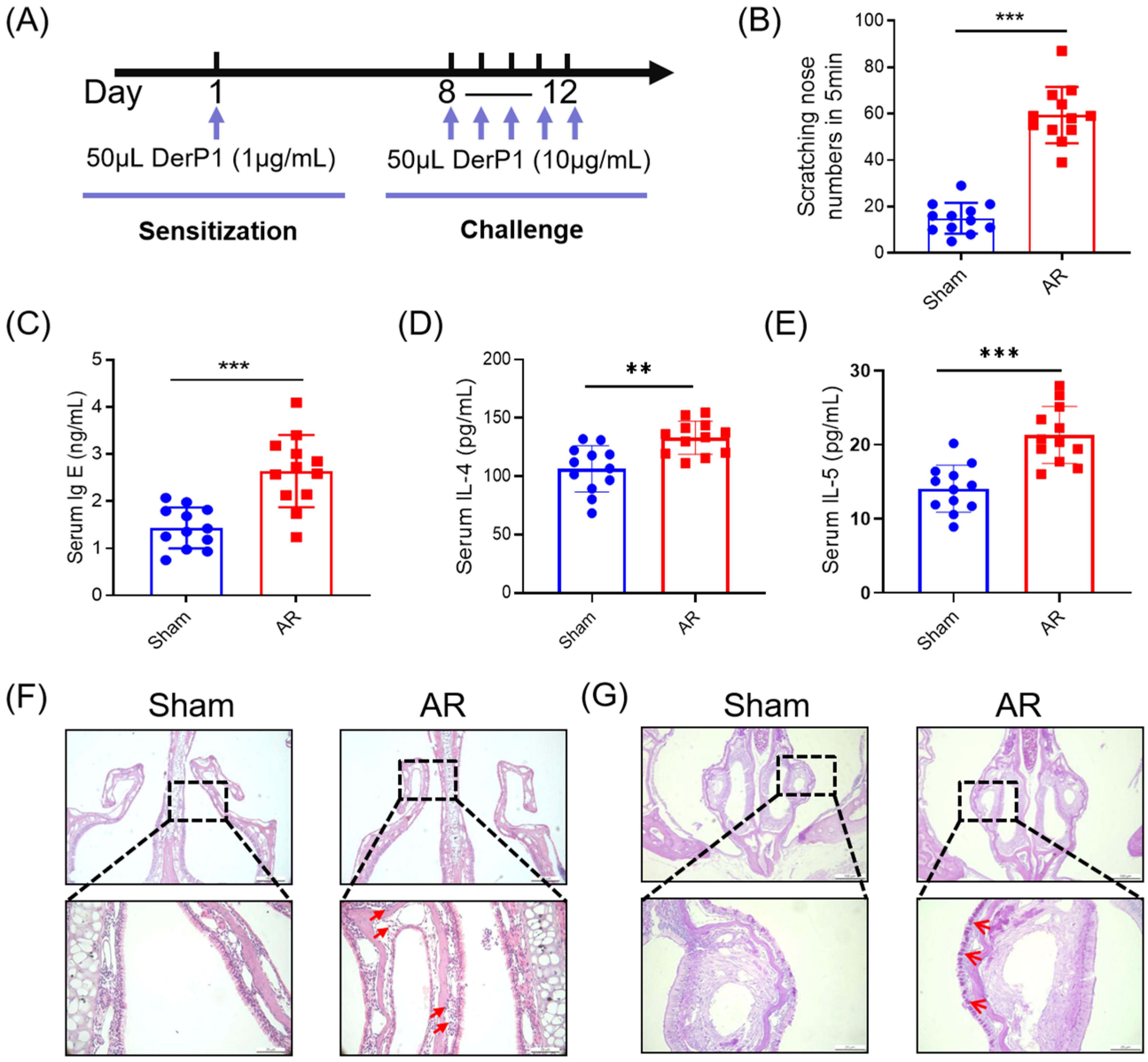

Figure 1 Successful evaluation of the DerP1-induced mouse AR model. (**A**) Paradigm and timeline of the AR mouse modeling design. (**B**) Statistical comparison of the number of nose scratching within 5 min in each group of mice. (**C**) Statistical comparison of serum total Ig E levels between groups using ELISA. (**D**) Statistical comparison of serum total IL-4 levels between groups using ELISA. (**E**) Statistical comparison of serum total IL-5 levels between groups using ELISA. (**F**) Histomorphologic changes of nasal mucosa under hematoxylin and eosin staining in each group of mice. (**G**) Histomorphologic changes of nasal mucosa under Periodic Acid-Schiff staining in each group of mice (the above graphs' scale bar = 100 μm, the below graphs' scale bar = 25 μm). Bar value = mean ± SEM; n = 3 animals per group for histopathological staining, n = 12 animals per group for nose scratching test and ELISA, and those graphs were analyzed using a *t*-test, $^{**}P < 0.01$, $^{***}P < 0.001$.

Histopathological testing revealed that mice in the AR group had lodged and shed cilia in the nasal mucosal epithelial layer. Additionally, the basement membrane was disordered, with the exudation of inflammatory cells and an increase in eosinophils and goblet cells (Figure 1F and G). All the above results confirm the successful construction of the AR mice model. TJs molecules are key connecting elements between epithelial cells, and their decreased expression is a critical indicator of epithelial barrier disruption. To examine alterations in the nasal mucosal epithelial barrier in AR mice, we employed qPCR, WB, and immunofluorescence to identify the TJ indicators, CLDN1 and OCLN. These findings indicate a significant reduction in the expression levels of these two signs, further implying that the nasal mucosal barrier in AR mice was impaired (Figure 2).

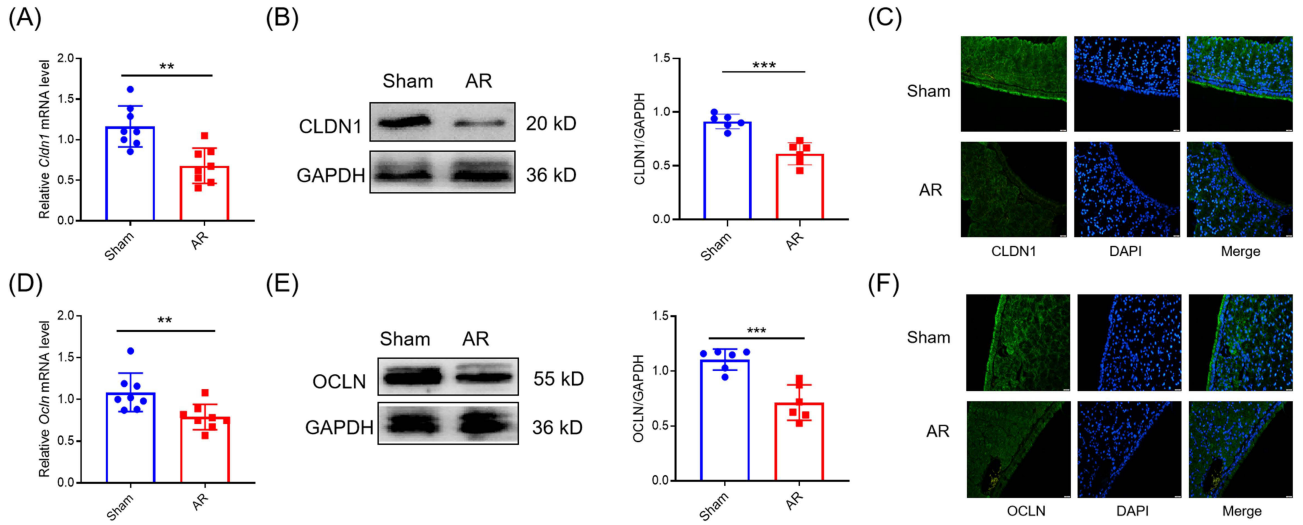

Figure 2 The changes of the nasal mucosal epithelial barrier in AR mice. (**A**) The changes of *Cldn1* mRNA expression level in the nasal mucosa of mice in each group using qPCR. (**B**) The changes of CLDN1 protein expression level in the nasal mucosa of mice in each group using WB. (**C**) IF detection of protein expression of CLDN1 in the nasal mucosal epithelium of mice in each group (×400, scale bar = 20 μm). (**D**) The changes of *Ocln* mRNA expression level in the nasal mucosa of mice in each group using qPCR. (**E**) The changes of OCLN protein expression level in the nasal mucosa of mice in each group using WB. (**F**) IF detection of protein expression of OCLN in the nasal mucosal epithelium of mice in each group (×400, scale bar = 20 μm). Bar value = mean ± SEM; n = 8 animals per group for qPCR, n = 6 animals per group for WB, n = 3 animals per group for IF. The graphs of qPCR and WB displayed were analyzed using a *t*-test, $^{**}P < 0.01$, $^{***}P < 0.001$.

IL-1β is Remarkably Associated with Epithelial Barrier Damage in the Nasal Mucosa of AR Mice

Our prior review indicated that IL-1β plays a critical role in the development, maintenance, and progression of AR.[25] Thus, we examined the expression levels of IL-1β and IL-1RA in the mouse nasal mucosa simultaneously. The results demonstrated that IL-1β was increased in the nasal mucosal tissues of AR mice, and there was an imbalance in the expression of IL-1β/IL-1RA (Figure 3A–D). According to previous studies, IL-1β could be linked to intestine and lung diseases that affect the mucosal epithelial barrier. While we proceeded to refine the correlation analysis, the findings revealed a strong negative correlation between the expression level of IL-1β and the changes in TJs molecules in the epithelial barrier of the mouse nasal mucosa (Figure 3E and F).

DerP1-Stimulated HNEpCs Exhibit a Decrease in TJs Molecules and a Rise in IL-1β

Considering that the in vivo experiments had more interfering factors, we selected HNEpCs for in vitro experiments to clarify changes in the nasal mucosal epithelial barrier after allergen stimulation. After 24 h of DerP1 stimulation of HNEpCs, there was a significant increase of IL-1β both within the cells and its secretion into the culture medium, accompanied by a decrease in intracellular IL-1RA (Figure 4A–E). Moreover, the indicators of TJs among HNEpCs were drastically reduced (Figure 4F–I), which was consistent with the results of the in vitro experiments.

IL-1β Directly Induces Damage to the HNEpCs Barrier

To summarize, upon activation with the allergen DerP1, nasal mucosal epithelial cells not only induce an imbalance between IL-1β and IL-1Ra but also cause damage to the epithelial barrier. To investigate whether IL-1β production is directly linked to the breaking of TJs among epithelial cells, we challenged HNEpCs simultaneously with humanized IL-1β or DerP1. The results demonstrated that the intervention of IL-1β can harm the intercellular tight junctions to an extent comparable to that of the allergen, both at the RNA and protein levels (Figure 5). Additional evidence supports the detrimental effects of IL-1β on the mucosal barrier.

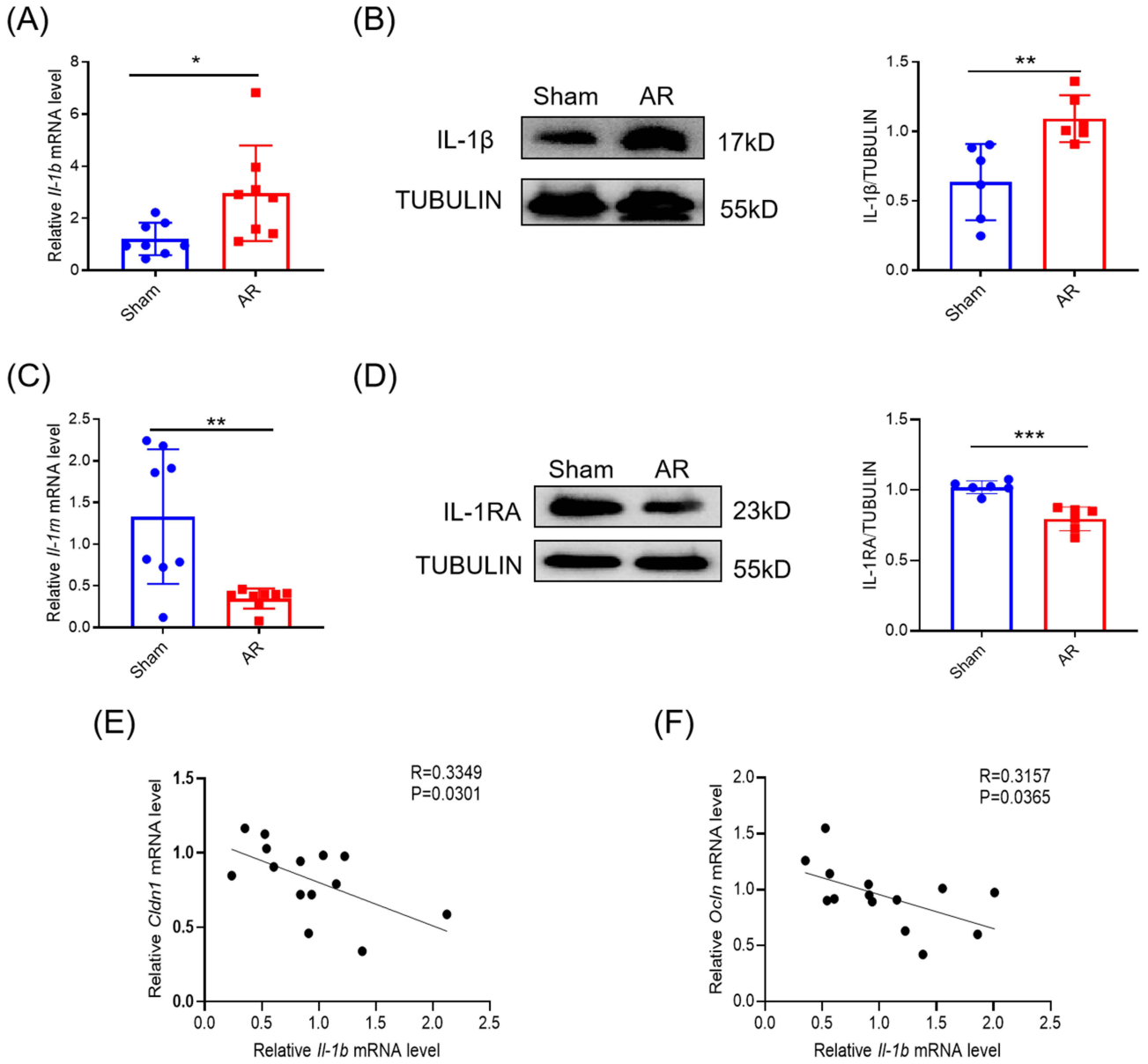

Figure 3 The changes of IL-1β/IL-1RA expression in the nasal mucosa of AR mice and the correlation between IL-1β and the changes of barrier index expression. (**A**) The changes of *Il-1b* mRNA expression level in the nasal mucosa of mice in each group using qPCR. (**B**) The changes of IL-1β protein expression level in the nasal mucosa of mice in each group using WB. (**C**) The changes of *Il-rn* mRNA expression level in the nasal mucosa of mice in each group using qPCR. (**D**) The changes of IL-1RA protein expression level in the nasal mucosa of mice in each group using WB. (**E**) The correlation analysis of the changes in the expression of *Il-b* and *Cldn1* in the nasal mucosa of mice. (**F**) The correlation analysis of the changes in the expression of *Il-b* and *Ocln* in the nasal mucosa of mice. Bar value = mean ± SEM; n = 8 animals per group for qPCR, n = 6 animals per group for WB, and n = 14 animals per group for correlation analysis. The graphs of qPCR and WB displayed were analyzed using a *t*-test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

The Binding of IL-1β and Its Receptor IL-1R1 Damages the Nasal Mucosal Epithelial Barrier

To verify the detrimental impact of IL-1β on the epithelial barrier, we pre-treated HNEpCs with Diacerein, an inhibitor that reduces the production of IL-1β, prior to allergen stimulation. The findings indicated that the use of Diacerein considerably reduced the damage to the TJ proteins CLDN1 and OCLN among HNEPCs (Figure 6A–D). As an important receptor in the IL-1 signaling pathway, IL-1β usually binds to IL-1R1 to exert its function. Subsequently, the IL-1R1 inhibitor, AF12198, successfully prevented DerP1-induced damage to the nasal mucosal epithelial barrier in

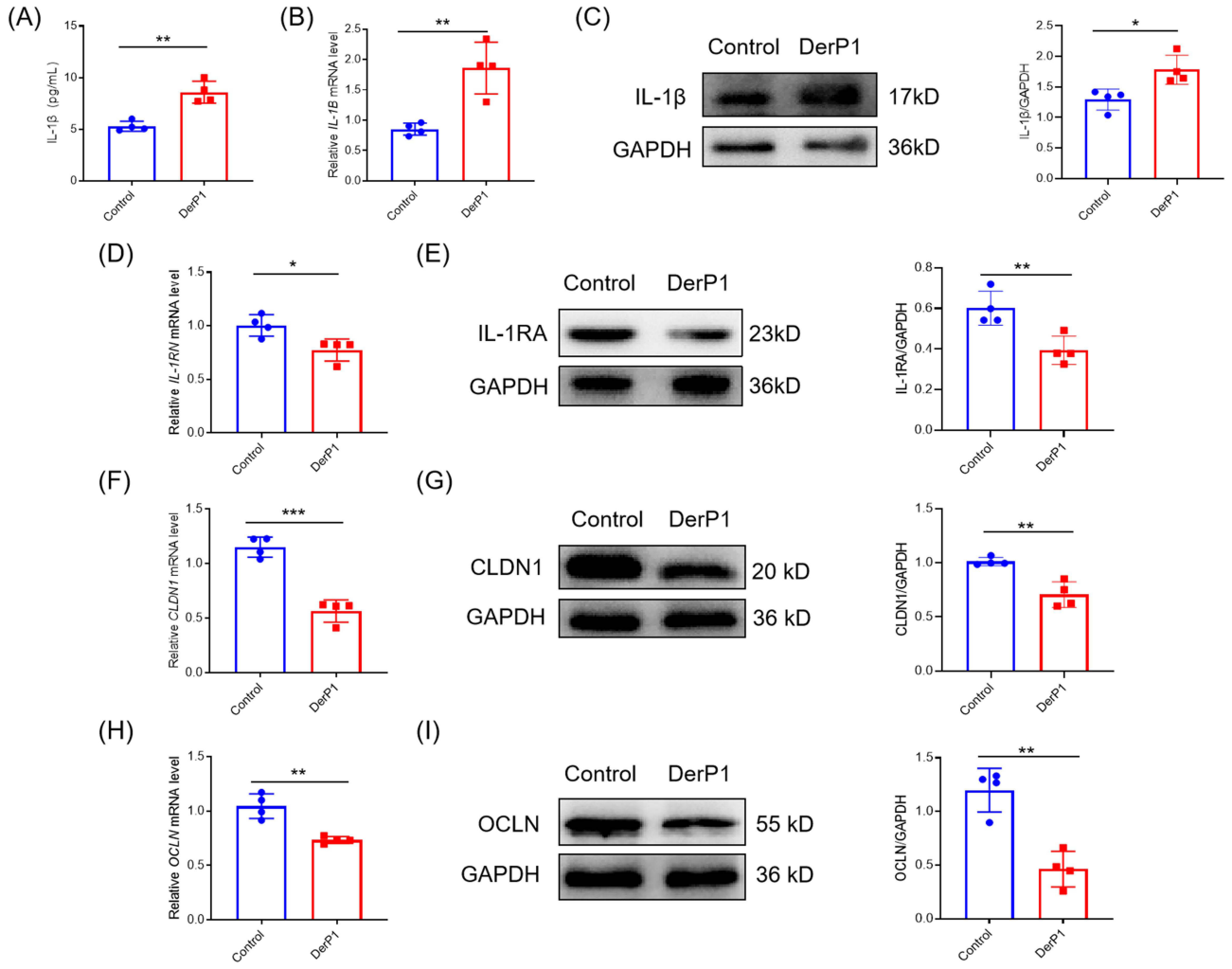

Figure 4 The changes of IL-1β/IL-1RA expression and effects on the epithelial barrier after DerP1 stimulation of HNEpCs. (**A**) The changes of IL-1β concentration in the cell culture medium of each group using ELISA. (**B**) The changes of *IL-1B* mRNA expression level in HNEpCs in each group using qPCR. (**C**) The changes of IL-1β protein expression level in HNEpCs in each group using WB. (**D**) The changes of *IL-1RN* mRNA expression level in HNEpCs in each group using qPCR. (**E**) The changes of IL-1RA protein expression level in HNEpCs in each group using WB. (**F**) The changes of *CLDN1* mRNA expression level in HNEpCs in each group using qPCR. (**G**) The changes of CLDN1 protein expression level in HNEpCs in each group using WB. (**H**) The changes of *OCLN* mRNA expression level in HNEpCs in each group using qPCR. (**I**) The changes of OCLN protein expression level in HNEpCs in each group using WB. Bar value = mean ± SEM; n = 4 per group for ELISA, qPCR, and WB. All graphs were analyzed using a *t*-test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

the cell intervention experiment (Figure 6E–H). These findings show that IL-1β, by binding to its receptor IL-1R1, is an essential factor in the damage to the nasal mucosal epithelial barrier in AR.

Discussion

With the changes in ecology and social environment, the incidence of AR continues to increase. In China, the incidence of AR has increased by 6.5% over the past decade. The accompanying symptoms and fatal asthma impose a huge burden on both the patient's family and society.[1,26] The trigger or aggravation of AR is mostly due to inhalation of allergens, such as house dust mites and pollen, in daily life. Of all the main allergens, house dust mites are the most prevalent and difficult to avoid. The AR caused by these can account for more than 50%, and in some areas, it can reach 80%.[27] Current research on the diagnosis and treatment of AR has mainly focused on Ig E- and Th2-type immune responses. New therapeutic targets are being continuously identified. However, for some patients with AR, especially refractory AR, there is no discernible therapeutic benefit. Advancements in electron microscopy have led to an increasing number of studies focusing on the close connection between allergic diseases and the epithelial barrier.[28–30] In 2011, Mattila et al suggested that allergic illnesses are epithelial barrier diseases.[31] Subsequent research has increasingly focused on the

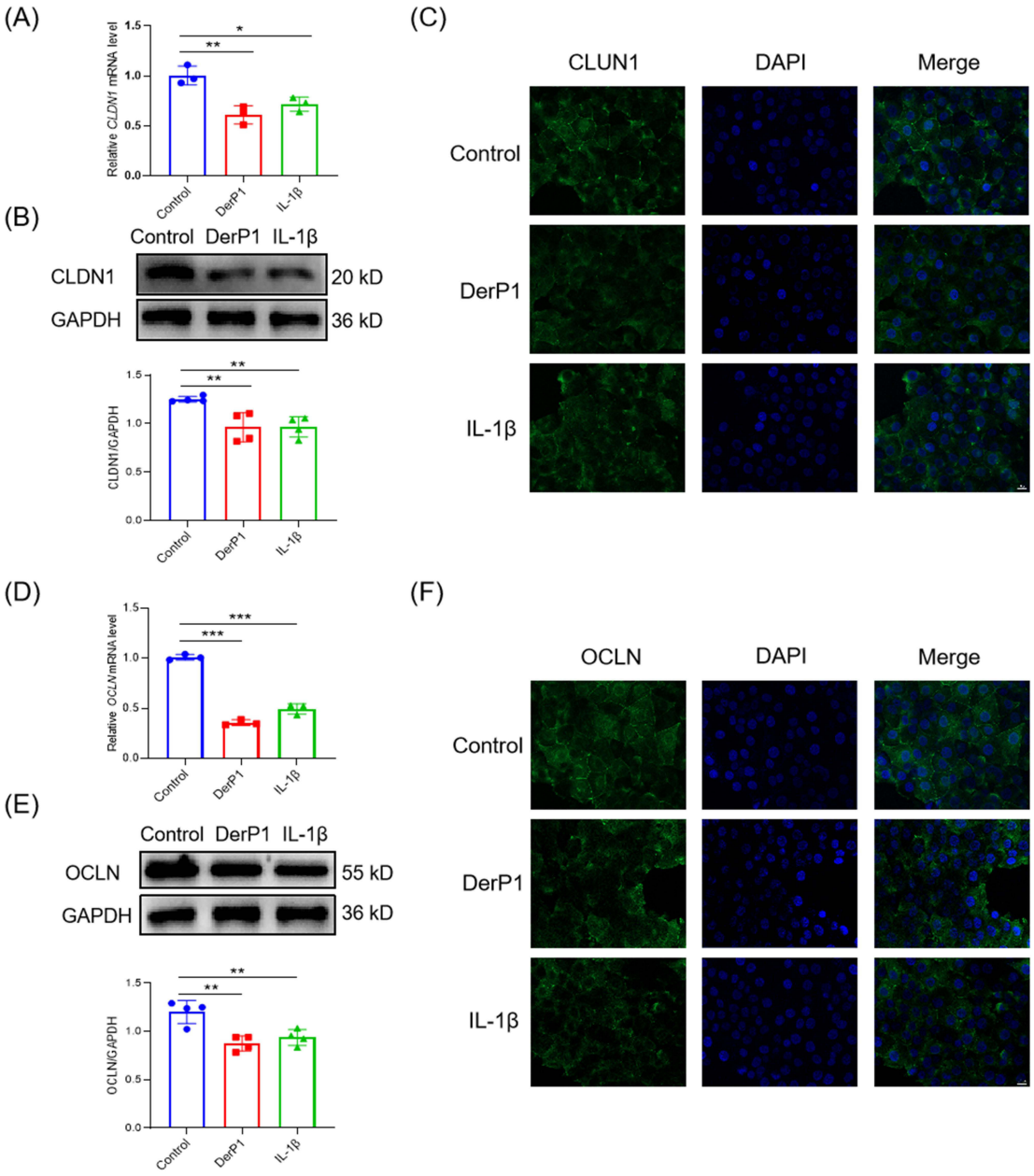

Figure 5 The changes of the epithelial barrier in HNEpCs after direct stimulation with human-derived IL-1β compared with DerP1 stimulation. (**A**) The changes of *CLDN1* mRNA expression level in HNEpCs in each group using qPCR. (**B**) The changes of CLDN1 protein expression level in HNEpCs in each group using WB. (**C**) The changes of CLDN1 protein expression level in HNEpCs in each group using IF (×400, scale bar = 20 μm). (**D**) The changes of *OCLN* mRNA expression level in HNEpCs in each group using qPCR. (**E**) The changes of OCLN protein expression level in HNEpCs in each group using WB. (**F**)The changes of OCLN protein expression level in HNEpCs in each group using IF (×400, scale bar = 20 μm). Bar value = mean ± SEM; n = 3 per group for qPCR and IF, n = 4 per group for WB. The graphs of qPCR and WB displayed were analyzed using one-way ANOVA (three groups). **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

fundamental functions of barrier defense and repair in allergic diseases, including allergic dermatitis and asthma.[32,33] Therefore, further research is urgently needed to explore the mechanisms of the nasal mucosal epithelial barrier and interventions for its treatment, which is considered a key direction for the prevention and treatment of AR.

The nasal mucosal epithelium serves as the starting point for breathing and olfaction in humans, as well as the initial site for responding to various physical and biological substances in the environment. To resist invasion by inhaled allergens, nasal mucosal epithelial cells are tightly connected to form the first defense barrier in the body, ensuring the stability of the internal environment. When the barrier is damaged, allergens continuously infiltrate, leading to sustained immune dysregulation within the nasal mucosa and promoting the development of allergic reactions.[34] While inhalant

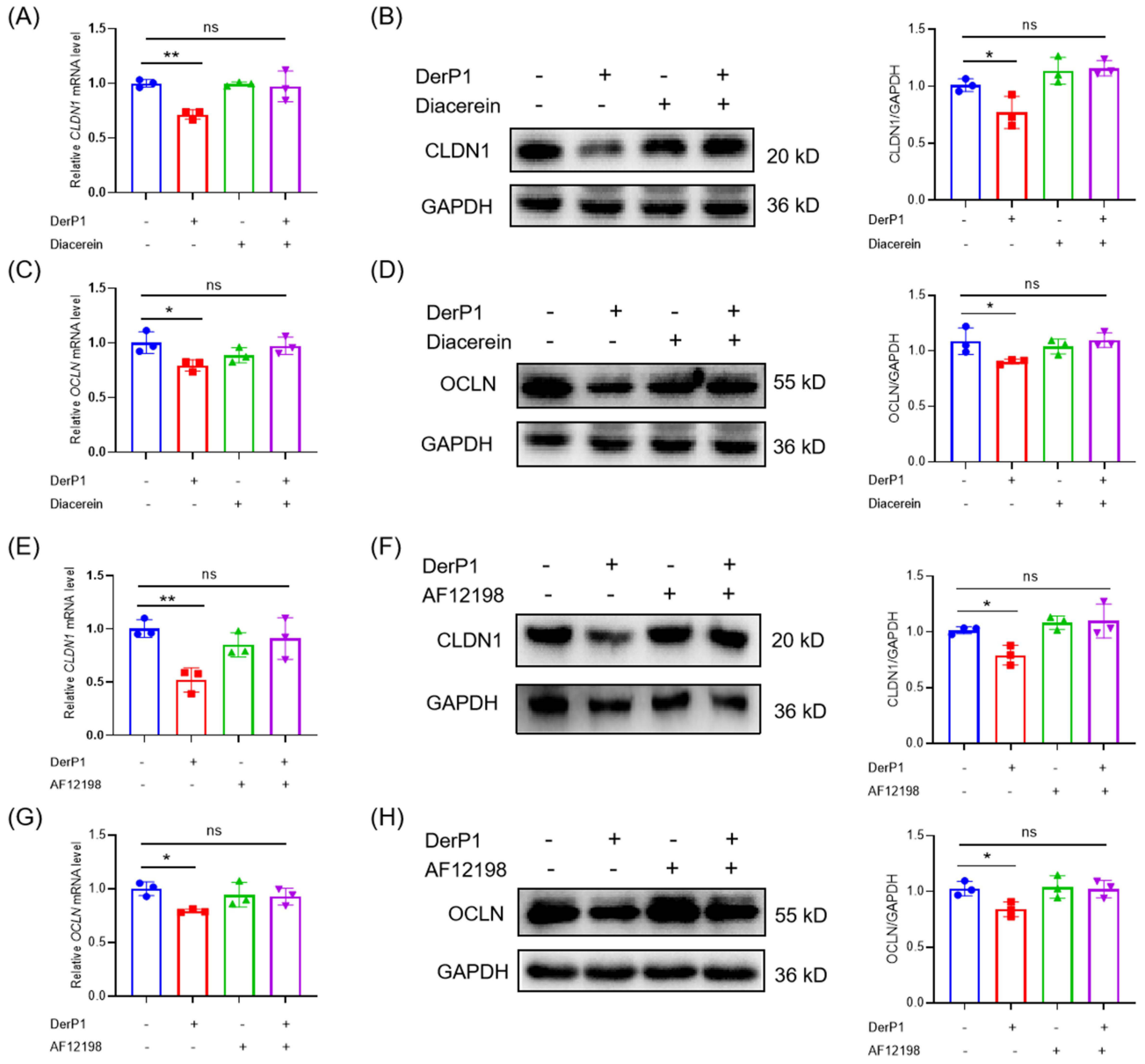

Figure 6 The effect of IL-1β inhibitor (Diacerein) and IL-1R1 inhibitor (AF12198) on the epithelial cell barrier of nasal mucosa after DerP1 stimulation. (**A**) The changes of *CLDN1* mRNA expression level in HNEpCs in each group using qPCR. (**B**) The changes of CLDN1 protein expression level in HNEpCs in each group using WB. (**C**) The changes of *OCLN* mRNA expression level in HNEpCs in each group using qPCR. (**D**) The changes of OCLN protein expression level in HNEpCs in each group using WB. (**E**) The changes of *CLDN1* mRNA expression level in HNEpCs in each group using qPCR. (**F**) The changes of CLDN1 protein expression level in HNEpCs in each group using WB. (**G**) The changes of *OCLN* mRNA expression level in HNEpCs in each group using qPCR. (**H**) The changes of OCLN protein expression level in HNEpCs in each group using WB. Bar value = mean±SEM; n = 3 per group for qPCR and WB. All graphs of qPCR and WB displayed were analyzed using one-way ANOVA (four groups). *$P < 0.05$, **$P < 0.01$, ns, not significant.

allergens destroy the integrity of the epithelial barrier, the production of certain inflammatory factors also impairs barrier function, and the formation of this vicious circle undoubtedly becomes the key to exacerbating the disease.[35]

TJs are located in the most apical region of cell-cell connections. They are composed of various transmembrane (CLDN family, OCLN, and junctional adhesion molecules) and intracellular (Zonula occludens family) proteins. The proteins CLDN1 and OCLN are at the forefront of TJs, spanning between cells and regulating the passage of large and small molecules as well as ions. Hence, they serve as crucial indicators of barrier integrity disruption.[36,37] In a previous study, it was found that one of the most important sensitizing proteins in house dust mites, DerP1, can break the airway epithelial barrier using its own proteases.[38] The results of our study also clarified the destructive effect of DerP1 on the nasal mucosal epithelial barrier. However, the specific mechanism through which it participates in the feedback loop of

barrier damage remains unclear. Effective protection or repair of the integrity of the nasal mucosal barrier to resist pathogen invasion is key to AR protection.

When allergens breach the mucosal epithelial barrier, various immune cells are stimulated to mount an immune response. IL-1β is highly sensitive to immune inflammation and can produce a substantial amount of pro-IL-1β in the early stages of the disease, then it is cleaved to release mature and active IL-1β from the cells, exerting subsequent biological effects.[39] Some of the activated IL-1β is released into tissues or the blood, targeting distant cells to mediate immune and inflammatory responses, while another portion acts on self or nearby cells through autocrine or paracrine forms.[39] Early studies have shown a significant increase in IL-1β levels in the blood, nasal secretions, and nasal mucosal tissues of patients with AR.[15,40,41] Moreover, there was a rise in IL-1β expression in both the in vitro and in vivo AR models using DerP1 as an allergen, and it was negatively correlated with the expression levels of TJs-related indicators. This finding implies that nasal mucosal epithelial cells produce a substantial amount of IL-1β during the pathogenesis of AR, which impacts the nasal mucosa's epithelial barrier function in some way and, therefore, accelerates the disease's physiological and pathological progression.

To investigate whether IL-1β generated by nasal mucosal epithelial cells serving as sentinel cells in allergic reactions impacts intercellular TJs via autocrine and paracrine pathways, we challenged ex vivo HNEpCs with IL-1β stimulation. The results further supported our hypothesis that IL-1β may be involved in the feedback loop mechanism of AR nasal mucosal barrier damage. In earlier studies, Rawat et al[42] demonstrated that IL-1β may cause the mucosal epithelial barrier to be disrupted in colitis by increasing the permeability of the small intestine mucosa. However, more attention has been paid to the role of IL-1β secreted by house dust mites as a key factor in pyroptosis within the inflammatory response to airway disease development.[43,44] However, its direct effect on the nasal mucosal epithelial barrier has not yet been investigated. Although Wu et al[45] found that the secretion of IL-1β may promote the repair of the intestinal mucosal barrier, and the specific intervention mechanism is still uncertain. At least for now, when we simulated the nasal mucosal epithelial environment under allergen stimulation, IL-1β at this concentration disrupted the nasal mucosal epithelial barrier. When we inhibited it with Diacerein (IL-1β inhibitor), the damage caused by DerP1 to the HNEpCs barrier was also significantly reduced.

Normally, IL-1β binds to its receptor, IL-1R1, to affect downstream consequences. Simultaneously, the body secretes IL-1RA, a corresponding antagonist, to compete with IL-1β for IL-1R1 binding. Based on the above, we also examined the expression changes of IL-1RA in mouse nasal mucosa and HNEpCs, and we discovered that IL-1RA was down-regulated in tandem with a rise in IL-1β. Although the changes in IL-1RA expression levels were not consistent in different studies, we considered that they were related to either the timing of detection or autoimmunization capacity.[46,47] In any case, the IL-1β/IL-1RA imbalance reminds us that intervention on the common receptor for both, IL-1R1, might also be a target of concern. So, we selected AF12198, an inhibitor of IL-1R1, to pretreat HNEpCs before DerP1 stimulation, and the results showed that blocking IL-1R1 was also able to alleviate the damage of the nasal mucosal epithelial barrier, which also indicated that IL-1β affected the nasal mucosal epithelial barrier through its receptor IL-1R1.

IL-1β is a critical inflammatory factor in the body's innate immunity and a pyroptosis-specific cytokine associated with inflammasome. The NOD-like receptor, as an essential player in triggering the assembly and activation of inflammasome as well as in promoting the maturation and secretion of IL-1β and IL-18, has also been repeatedly shown to have a potential role in airway diseases.[48–50] NLRP3, the factor of greatest interest, has also been shown to promote the release of IL-1β and IL-18 via an increase in CASP1 in the AR, which in turn induces localized allergic inflammation.[16] This was also validated in our in vitro experiments. DerP1 stimulation increase both IL-1β and IL-18 levels, but the changes in IL-1β are more significant. Moreover, the inhibition of CASP1 also reduced IL-1β more effectively (Supplementary Figure 1). Additionally, the "inflammasome/Th17/neutrophil axis cytokine/IL-1β" may also temporarily impair epithelial barrier function.[51] The above studies all suggest that it is feasible to conduct in-depth research by considering IL-1β as an "endpoint factor". Although the intervention of its upstream indicators can reduce the expression of IL-1β and alleviate the damage to the nasal mucosal barrier in AR to a certain extent,[43,52] in clinical practice, IL-1β levels are already elevated when we contact AR patients. Compared with the preceding indicators, targeting IL-1β seems to be a better way to achieve the clinical expectation of "early detection, early diagnosis, early

treatment" as well as to curb the disease at the "starting point" (ie, the nasal mucosal epithelial barrier). Therefore, we aimed to identify potential therapeutic targets and strategies to prevent damage to the nasal mucosal epithelial barrier in patients with AR.

The current research also suggests that the role of IL-1β in AR is not limited to nasal mucosal epithelial cells, but it also assists the development of hypersensitivity in allergic airway diseases by promoting histamine release from mast cells,[53] enhancing Th2-type immune responses,[54] and so on. During stimulation of CD4+ T cells with IL-1β, a significant increase in the secretion of the type 2 inflammatory factors IL-4, IL-5, and IL-13 was observed, and this effect can be effectively suppressed when the IL-1R receptor on T cells was inhibited (Supplementary Figure 2). This further indicates that IL-1β plays an important role in allergic diseases. Thus, although our study primarily focused on the effects of IL-1β at the mucosal epithelial barrier, its roles in AR are diverse and indispensable, and the specific blockade of IL-1β is essential for the treatment of AR. At present, drugs targeting IL-1β, such as the IL-1R antagonist (Anakinra, Kineret®), the IL-1 trapping agent (Rrilonacept, Regeneron®), and the human anti-IL-1β monoclonal antibody (Canakinumab, Ilaris®) are used more for systemic immunoinflammatory diseases, and their scope of application does not include AR. However, the animal experiments performed by Ritter et al[55] and Hernandez et al[56] showed the promise of IL-1β-targeted drugs for the treatment of allergic airway diseases. Our study only pharmacologically investigated these mechanisms in ex vivo cells. A more comprehensive experimental validation in the future with mice specifically knocked down for IL-1R1 in nasal mucosal epithelial cells, or the development of targeted agents restricted to intranasal administration might be better for the discovery and clinical translation of relevant clinical drugs for AR.

In conclusion, combined with our findings and previous reports, IL-1β has an important role in the pathogenesis of AR (Figure 7), which we summarize below: (1) Initial stage: After allergen stimulation of nasal mucosal epithelial cells, IL-1β is produced and binds to IL-1R1 receptors in the epithelial cells or in adjacent epithelial cells. This promotes the destruction of TJs and hinders the function of the epithelial barrier, resulting in persistent damage to the lesions at the initial site of the disease. (2) Progress stage: Following the invasion of allergens into the tissue, IL-1β produced by nasal

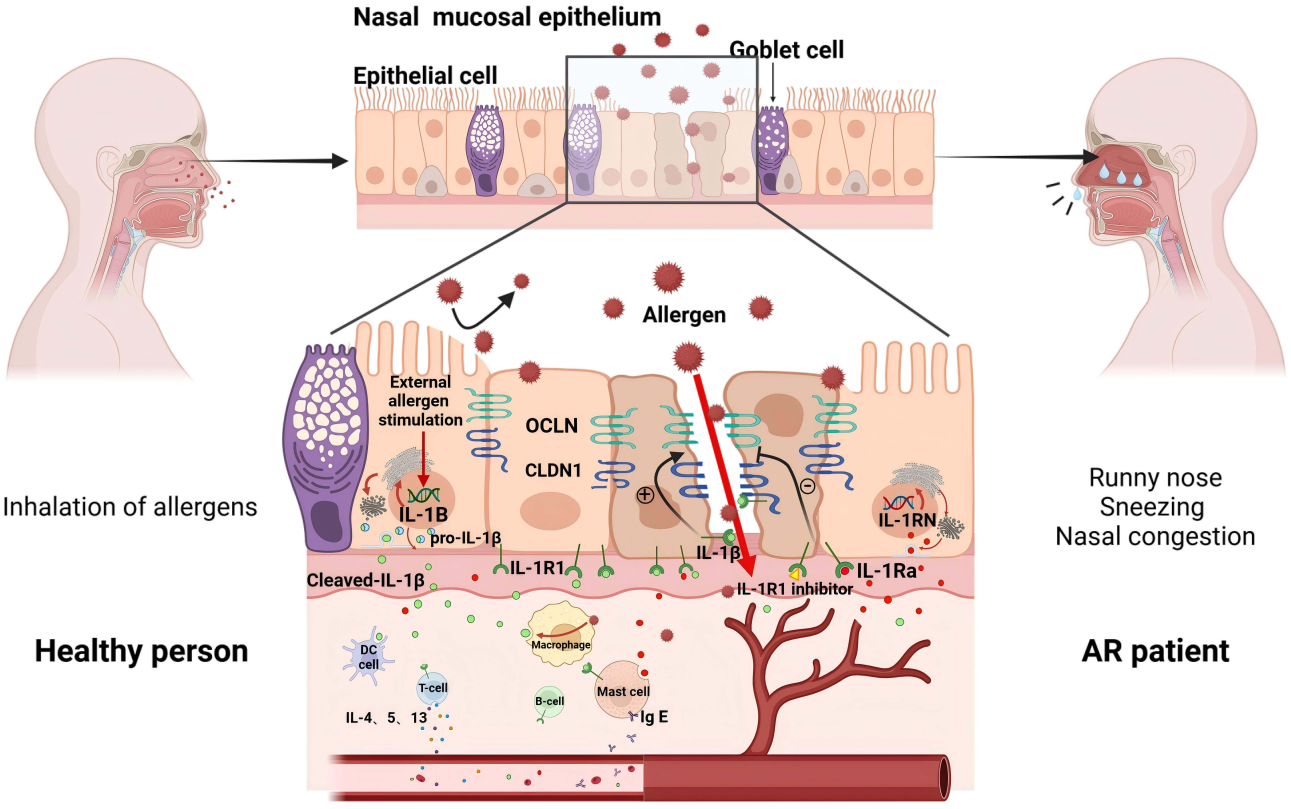

Figure 7 The possible mechanism of action of IL-1β on the nasal mucosal epithelial barrier in AR.

mucosal epithelial cells and tissues acts on various lymphocytes and mast cells, promoting Th2-type immune response and triggering the release of Ig E and histamine, thus advancing the development of allergic reactions. (3) Feedback stage: When illness is present, an increase in IL-1β causes an imbalance in the IL-1β/IL-1RA. It leads the body to manufacture more IL-1RA, which binds to the IL-1R1 receptor in a competitive manner with pro-inflammatory IL-1β, regulating the immune-inflammatory response. As a result, intervening in or blocking the beginning stage of the disease from its origin is an extremely vital point and critical time to achieve efficient prevention and treatment. The pathogenesis of AR is not a single factor or a localized effect but an intricate and comprehensive response of the organism, which needs to be explored in a deeper and broader way.

Conclusions

In summary, IL-1β can reduces the expression of TJs molecules CLDN1 and OCLN in the nasal mucosal epithelial barrier in AR. Preventing IL-1β or its receptor is an efficient way to protect the nasal mucosal barrier. It is possible that IL-1β will emerge as a potential therapeutic target for AR.

Abbreviations

AR, allergic rhinitis; TJs, tight junctions; IL-1β, interleukin-1β; CASP1, caspase 1; CLDN1, claudin-1; DerP1, Dermatophagoides pteronyssinus 1; HNEpCs, human nasal epithelial cells; ELISA, enzyme-linked immunosorbent assay; IF, immunofluorescence; OCLN, occludin; RT-qPCR, quantitative real-time polymerase chain reaction; WB, Western blot analysis; SEM, standard error of the mean; Ig E, immunoglobulin E.

Data Sharing Statement

The datasets supporting the conclusions of this article are included within the article.

Ethics Approval and Informed Consent

Animal purchasing, feeding, and subsequent experiments were approved by the Ethics Committee of Yantai Yuhuangding Hospital (2022-104).

Consent for Publication

All authors have confirmed and agreed to publish this manuscript.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflicts of interest.

References

1. Brożek JL, Bousquet J, Agache I, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines-2016 revision. *J Allergy Clin Immunol*. 2017;140(4):950–958. doi:10.1016/j.jaci.2017.03.050
2. Fukuoka A, Yoshimoto T. Barrier dysfunction in the nasal allergy. *Allergol Int*. 2018;67(1):18–23. doi:10.1016/j.alit.2017.10.006
3. Dong X, Ding M, Zhang J, et al. Involvement and therapeutic implications of airway epithelial barrier dysfunction in type 2 inflammation of asthma. *Chin Med J*. 2022;135(5):519–531. doi:10.1097/CM9.0000000000001983
4. Kortekaas Krohn I, Seys SF, Lund G, et al. Nasal epithelial barrier dysfunction increases sensitization and mast cell degranulation in the absence of allergic inflammation. *Allergy*. 2020;75(5):1155–1164. doi:10.1111/all.14132
5. Hammad H, Lambrecht BN. Barrier epithelial cells and the control of type 2 immunity. *Immunity*. 2015;43(1):29–40. doi:10.1016/j.immuni.2015.07.007
6. Luissint AC, Parkos CA, Nusrat A. Inflammation and the intestinal barrier: leukocyte-epithelial cell interactions, cell junction remodeling, and mucosal repair. *Gastroenterology*. 2016;151(4):616–632. doi:10.1053/j.gastro.2016.07.008
7. Pothoven KL, Schleimer RP. The barrier hypothesis and Oncostatin M: restoration of epithelial barrier function as a novel therapeutic strategy for the treatment of type 2 inflammatory disease. *Tissue Barriers*. 2017;5(3):e1341367. doi:10.1080/21688370.2017.1341367
8. Nur Husna SM, Tan HT, Md Shukri N, Mohd Ashari NS, Wong KK. Nasal epithelial barrier integrity and tight junctions disruption in allergic rhinitis: overview and pathogenic insights. *Front Immunol*. 2021;12:663626. doi:10.3389/fimmu.2021.663626
9. Akdis CA. Does the epithelial barrier hypothesis explain the increase in allergy, autoimmunity and other chronic conditions? *Nat Rev Immunol*. 2021;21(11):739–751. doi:10.1038/s41577-021-00538-7
10. Dinarello CA. Immunological and inflammatory functions of the interleukin-1 family. *Annu Rev Immunol*. 2009;27:519–550. doi:10.1146/annurev.immunol.021908.132612
11. Gabay C, Lamacchia C, Palmer G. IL-1 pathways in inflammation and human diseases. *Nat Rev Rheumatol*. 2010;6(4):232–241. doi:10.1038/nrrheum.2010.4
12. Iype J, Odermatt A, Bachmann S, Coeudevez M, Fux M. IL-1β promotes immunoregulatory responses in human blood basophils. *Allergy*. 2021;76(7):2017–2029. doi:10.1111/all.14760
13. Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunol Rev*. 2018;281(1):8–27. doi:10.1111/imr.12621
14. Rosenwasser LJ. Biologic activities of IL-1 and its role in human disease. *J Allergy Clin Immunol*. 1998;102(3):344–350. doi:10.1016/S0091-6749(98)70118-6
15. Han MW, Kim SH, Oh I, Kim YH, Lee J. Serum IL-1β can be a biomarker in children with severe persistent allergic rhinitis. *Allergy Asthma Clin Immunol*. 2019;15:58. doi:10.1186/s13223-019-0368-8
16. Zhou H, Zhang W, Qin D, et al. Activation of NLRP3 inflammasome contributes to the inflammatory response to allergic rhinitis via macrophage pyroptosis. *Int Immunopharmacol*. 2022;110:109012. doi:10.1016/j.intimp.2022.109012
17. Qiao YL, Zhu MW, Xu S, et al. Allergen-induced CD11c + dendritic cell pyroptosis aggravates allergic rhinitis. *Cell Commun Signal*. 2023;21(1):281. doi:10.1186/s12964-023-01309-8
18. Radzikowska U, Eljaszewicz A, Tan G, et al. Rhinovirus-induced epithelial RIG-I inflammasome suppresses antiviral immunity and promotes inflammation in asthma and COVID-19. *Nat Commun*. 2023;14(1):2329. doi:10.1038/s41467-023-37470-4
19. Yao Y, Yang YJ, Zhang Y, Song XC. Mechanism of IL-1β in upper airway inflammatory diseases. *J Otolaryngol Ophthalmol Shandong Univ*. 2022;36:130–135. (in Chinese).
20. Wei-xu H, Qin X, Zhu W, et al. Therapeutic potential of anti-IL-1β IgY in Guinea pigs with allergic asthma induced by ovalbumin. *Mol Immunol*. 2014;58(1):139–149. doi:10.1016/j.molimm.2013.11.006
21. Willart MA, Deswarte K, Pouliot P, et al. Interleukin-1α controls allergic sensitization to inhaled house dust mite via the epithelial release of GM-CSF and IL-33. *J Exp Med*. 2012;209(8):1505–1517. doi:10.1084/jem.20112691
22. Kaminsky LW, Al-Sadi R, Ma TY. IL-1β and the intestinal epithelial tight junction barrier. *Front Immunol*. 2021;12:767456. doi:10.3389/fimmu.2021.767456
23. Matarazzo L, Hernandez Santana YE, Walsh PT, Fallon PG. The IL-1 cytokine family as custodians of barrier immunity. *Cytokine*. 2022;154:155890. doi:10.1016/j.cyto.2022.155890
24. Steelant B, Farré R, Wawrzyniak P, et al. Impaired barrier function in patients with house dust mite-induced allergic rhinitis is accompanied by decreased occludin and zonula occludens-1 expression. *J Allergy Clin Immunol*. 2016;137(4):1043–1053. doi:10.1016/j.jaci.2015.10.050
25. Wang HR, Wei SZ, Song XY, et al. IL-1β and allergy: focusing on its role in allergic rhinitis. *Mediators Inflamm*. 2023;2023:1265449. doi:10.1155/2023/1265449
26. Zhang Y, Lan F, Zhang L. Advances and highlights in allergic rhinitis. *Allergy*. 2021;76(11):3383–3389. doi:10.1111/all.15044
27. Chinese Collaborative Group on Allergic Rhinitis Research. Expert consensus on subcutaneous immunotherapy for allergic rhinitis 2015. *Chin Arch Otolaryngol Head Neck Surg*. 2015;22:379–404. (in Chinese).
28. Celebi Sozener Z, Ozdel Ozturk B, Cerci P, et al. Epithelial barrier hypothesis: effect of the external exposome on the microbiome and epithelial barriers in allergic disease. *Allergy*. 2022;77(5):1418–1449. doi:10.1111/all.15240
29. Goleva E, Berdyshev E, Leung DY. Epithelial barrier repair and prevention of allergy. *J Clin Invest*. 2019;129(4):1463–1474. doi:10.1172/JCI124608
30. Hellings PW, Steelant B. Epithelial barriers in allergy and asthma. *J Allergy Clin Immunol*. 2020;145(6):1499–1509. doi:10.1016/j.jaci.2020.04.010
31. Mattila P, Joenväärä S, Renkonen J, Toppila-Salmi S, Renkonen R. Allergy as an epithelial barrier disease. *Clin Transl Allergy*. 2011;1(1):5. doi:10.1186/2045-7022-1-5
32. Heijink IH, Kuchibhotla VNS, Roffel MP, et al. Epithelial cell dysfunction, a major driver of asthma development. *Allergy*. 2020;75(8):1902–1917. doi:10.1111/all.14421
33. van den Bogaard EH, Elias PM, Goleva E, et al. Targeting skin barrier function in atopic dermatitis. *J Allergy Clin Immunol Pract*. 2023;11(5):1335–1346. doi:10.1016/j.jaip.2023.02.005
34. Duan S, Han XL, Li Y, Zhang L. Research on the expression of tight junctions on allergic rhinitis. *Chin Arch Otolaryngol Head Neck Surg*. 2021;28:293–296. (in Chinese).

35. Georas SN, Rezaee F. Epithelial barrier function: at the front line of asthma immunology and allergic airway inflammation. *J Allergy Clin Immunol*. 2014;134(3):509–520. doi:10.1016/j.jaci.2014.05.049
36. Kojima T, Go M, Takano K, et al. Regulation of tight junctions in upper airway epithelium. *Biomed Res Int*. 2013;2013:947072. doi:10.1155/2013/947072
37. Shen L, Weber CR, Raleigh DR, Yu D, Turner JR. Tight junction pore and leak pathways: a dynamic duo. *Annu Rev Physiol*. 2011;73:283–309. doi:10.1146/annurev-physiol-012110-142150
38. Wan H, Winton HL, Soeller C, et al. Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. *J Clin Invest*. 1999;104(1):123–133. doi:10.1172/JCI5844
39. Lopez-Castejon G, Brough D. Understanding the mechanism of IL-1β secretion. *Cytokine Growth Factor Rev*. 2011;22(4):189–195. doi:10.1016/j.cytogfr.2011.10.001
40. Jeong HJ, Shin SY, Oh HA, Kim MH, Cho JS, Kim HM. IL-32 up-regulation is associated with inflammatory cytokine production in allergic rhinitis. *J Pathol*. 2011;224(4):553–563. doi:10.1002/path.2899
41. Sim TC, Grant JA, Hilsmeier KA, Fukuda Y, Alam R. Proinflammatory cytokines in nasal secretions of allergic subjects after antigen challenge. *Am J Respir Crit Care Med*. 1994;149(2 Pt 1):339–344. doi:10.1164/ajrccm.149.2.8306027
42. Rawat M, Nighot M, Al-Sadi R, et al. IL1B increases intestinal tight junction permeability by up-regulation of MIR200C-3p, which degrades occludin mRNA. *Gastroenterology*. 2020;159(4):1375–1389. doi:10.1053/j.gastro.2020.06.038
43. Ge X, Cai F, Shang Y, et al. PARK2 attenuates house dust mite-induced inflammatory reaction, pyroptosis and barrier dysfunction in BEAS-2B cells by ubiquitinating NLRP3. *Am J Transl Res*. 2021;13(1):326–335.
44. Tsai YM, Chiang KH, Hung JY, et al. Der f1 induces pyroptosis in human bronchial epithelia via the NLRP3 inflammasome. *Int J Mol Med*. 2018;41(2):757–764. doi:10.3892/ijmm.2017.3310
45. Wu WH, Kim M, Chang LC, et al. Interleukin-1β secretion induced by mucosa-associated gut commensal bacteria promotes intestinal barrier repair. *Gut Microbes*. 2022;14(1):2014772. doi:10.1080/19490976.2021.2014772
46. Krouse HJ, Davis JE, Krouse JH. Immune mediators in allergic rhinitis and sleep. *Otolaryngol Head Neck Surg*. 2002;126(6):607–613. doi:10.1067/mhn.2002.125300
47. Wagenmann M, Schumacher L, Bachert C. The time course of the bilateral release of cytokines and mediators after unilateral nasal allergen challenge. *Allergy*. 2005;60(9):1132–1138. doi:10.1111/j.1398-9995.2005.00867.x
48. Månsson A, Bogefors J, Cervin A, Uddman R, Cardell LO. NOD-like receptors in the human upper airways: a potential role in nasal polyposis. *Allergy*. 2011;66(5):621–628. doi:10.1111/j.1398-9995.2010.02527.x
49. Bogefors J, Rydberg C, Uddman R, et al. Nod1, Nod2 and Nalp3 receptors, new potential targets in treatment of allergic rhinitis? *Allergy*. 2010;65(10):1222–1226. doi:10.1111/j.1398-9995.2009.02315.x
50. Zhou H, Wang L, Lv W, Yu H. The NLRP3 inflammasome in allergic diseases: mechanisms and therapeutic implications. *Clin Exp Med*. 2024;24(1):231. doi:10.1007/s10238-024-01492-z
51. Tan HT, Hagner S, Ruchti F, et al. Tight junction, mucin, and inflammasome-related molecules are differentially expressed in eosinophilic, mixed, and neutrophilic experimental asthma in mice. *Allergy*. 2019;74(2):294–307. doi:10.1111/all.13619
52. Yang Z, Liang C, Wang T, et al. NLRP3 inflammasome activation promotes the development of allergic rhinitis via epithelium pyroptosis. *Biochem Biophys Res Commun*. 2020;522(1):61–67. doi:10.1016/j.bbrc.2019.11.031
53. Subramanian N, Bray MA. Interleukin 1 releases histamine from human basophils and mast cells in vitro. *J Immunol*. 1987;138(1):271–275. doi:10.4049/jimmunol.138.1.271
54. Nakae S, Komiyama Y, Yokoyama H, et al. IL-1 is required for allergen-specific Th2 cell activation and the development of airway hypersensitivity response. *Int Immunol*. 2003;15(4):483–490. doi:10.1093/intimm/dxg054
55. Ritter M, Straubinger K, Schmidt S, et al. Functional relevance of NLRP3 inflammasome-mediated interleukin (IL)-1β during acute allergic airway inflammation. *Clin Exp Immunol*. 2014;178(2):212–223. doi:10.1111/cei.12400
56. Hernandez ML, Mills K, Almond M, et al. IL-1 receptor antagonist reduces endotoxin-induced airway inflammation in healthy volunteers. *J Allergy Clin Immunol*. 2015;135(2):379–385. doi:10.1016/j.jaci.2014.07.039