Impaired barrier function in patients with house dust mite–induced allergic rhinitis is accompanied by decreased occludin and zonula occludens-1 expression

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Background: Tight junction (TJ) defects have recently been associated with asthma and chronic rhinosinusitis. The expression, function, and regulation of nasal epithelial TJs remain unknown in patients with allergic rhinitis (AR).
Objective: We investigated the expression, function, and regulation of TJs in the nasal epithelium of patients with house dust mite (HDM)–induced AR and in an HDM-induced murine model of allergic airway disease.
Methods: Air-liquid interface cultures of primary nasal epithelial cells of control subjects and patients with HDM-induced AR were used for measuring transepithelial resistance and passage to fluorescein isothiocyanate–dextran 4 kDa (FD4). *Ex vivo* transtissue resistance and FD4 permeability of nasal mucosal explants were measured. TJ expression was evaluated by using real-time quantitative PCR and immunofluorescence. In addition, the effects of IL-4, IFN-γ, and fluticasone propionate (FP) on nasal epithelial cells were investigated *in vitro*. An HDM murine model was used to study the effects of allergic inflammation and FP treatment on transmucosal passage of FD4 *in vivo*.
Results: A decreased resistance *in vitro* and *ex vivo* was found in patients with HDM-induced AR, with increased FD4 permeability and reduced occludin and zonula occludens-1 expression. AR symptoms correlated inversely with resistance in patients with HDM-induced AR. *In vitro* IL-4 decreased transepithelial resistance and increased FD4 permeability, whereas IFN-γ had no effect. FP prevented IL-4–induced barrier dysfunction *in vitro*. In an HDM murine model FP prevented the allergen-induced increased mucosal permeability.
Conclusion: We found impaired nasal epithelial barrier function in patients with HDM-induced AR, with lower occludin and zonula occludens-1 expression. IL-4 disrupted epithelial integrity *in vitro*, and FP restored barrier function. Better understanding of nasal barrier regulation might lead to a better understanding and treatment of AR. (J Allergy Clin Immunol 2016;137:1043-53.)

Key words: *Allergic rhinitis, tight junctions, fluticasone propionate, IL-4, leak pathway, epithelial resistance, epithelial permeability*

A critical role of the airway epithelium is forming a physical barrier protecting the body from inhaled harmful substances. The mucosal barrier is primarily determined by the integrity of intercellular junctions through which epithelial cells are connected to each other (ie, tight junctions [TJs], adherens junctions, and desmosomes), ultimately sealing off the paracellular space.[1] TJs form the most apical intercellular junction between epithelial cells, providing functional polarity between the apical and basolateral domains.[2] TJs consist of different transmembrane proteins, including occludin, tricellulin, the claudin family, and junctional

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Abbreviations used
ALI: Air-liquid interface
AR: Allergic rhinitis
FD4: Fluorescein isothiocyanate–dextran 4 kDa
FP: Fluticasone propionate
HDM: House dust mite
INS: Intranasal steroid
NEC: Nasal epithelial cell
RT-qPCR: Real-time quantitative PCR
TER: Transepithelial resistance
TJ: Tight junction
ZO: Zonula occludens

adhesion molecules.[3] They form intercellular homodimers/heterodimers between neighboring cells, limiting the paracellular passage of macromolecules. Scaffold adaptor proteins, such as cingulin and the zonula occludens (ZO) family, connect the transmembrane proteins to the actin cytoskeleton. TJs are involved in 2 transport pathways: pore and leak pathways.[4] The leak pathway is mainly regulated by occludin and ZO, allowing limited passage of larger molecules, whereas the pore pathway is a size- and charge-selective high-capacity route for ions, as regulated by claudins.

Disturbed TJ function, expression, or both can lead to the entrance of foreign pathogens, irritants, and allergens into the organism.[5] Multiple disorders, such as asthma,[6] inflammatory bowel disease,[7] functional dyspepsia,[8] and atopic dermatitis,[9] have been linked to defective or altered TJ function. Recently, impaired epithelial barrier function was found in patients with chronic rhinosinusitis with or without nasal polyps,[10] suggesting changes in TJ arrangement in the sinonasal mucosa. Nevertheless, the function, expression, and regulation of TJs have not been investigated in patients with allergic rhinitis (AR).

AR is defined as an inflammation of the nasal mucosa resulting from an IgE-mediated allergy to inhaled allergens.[11] AR affects 30% of the Western population and causes symptoms, such as nasal obstruction, rhinorrhea, sneezing, and itchy nose.[12] Despite the available treatment options, such as intranasal steroids (INSs), antihistamines, or leukotriene receptor antagonists and immunotherapy,[13,14] 20% of patients with AR do not respond properly to treatment, representing a therapeutic challenge with a large socioeconomic burden.[15-18] The reason for lack of response to treatment is multifactorial, with barrier dysfunction being one of the reasons. A dysfunctional epithelial barrier might give rise to enhanced uptake of allergens and exogenous particles, leading to more activation of mast cells and nerve fibers.[5,19] Therefore it is of great interest to study epithelial barrier and TJ function in patients with AR.

This study aimed at exploring the function, expression, and regulation of TJs in the nasal epithelium of patients with house dust mite (HDM)–induced AR. Using human air-liquid interface (ALI) cultures of primary nasal epithelial cells (NECs), mucosal explants, and an HDM-induced murine model of allergic airway disease, we demonstrate an impaired barrier in patients with AR and a barrier-correcting effect of fluticasone propionate (FP). FP restored barrier integrity, which might be associated with promotion of expression of occludin and ZO-1.

METHODS

Patients

For *in vitro* analyses, NECs were isolated from the inferior turbinates of control subjects (n = 16) and steroid-naive patients with HDM-induced AR (n = 9) during aesthetic and/or functional rhinoplasty. Control subjects were nonsmokers, did not report any nasal symptoms suggestive of AR, and did not have a history of AR or rhinosinusitis. All included patients with HDM-induced AR were symptomatic (≥2 nasal symptoms of AR) at the time of inclusion, were nonsmoking nonasthmatic subjects, and did not show any clinical sign of infection in the nose at time of surgery. Patient information can be found in Table I.

Nasal biopsy specimens were taken from 10 healthy nonallergic control subjects without any nasal symptoms and from 15 patients with HDM-induced AR to evaluate epithelial barrier integrity at the mucosal explant level. Biopsy specimens were taken 10 minutes after application of a spray with local anesthesia (5% cocaine). Four nasal biopsy specimens were taken with a Fokkens forceps. The biopsy specimens were kept on ice in saline for further analysis. Patients' demographics can be found in Table II.

The study protocol was approved by the local ethics committee of UZ Leuven. The study was registered at clinicaltrial.gov (NCT02461797).

Evaluation of nasal symptoms

Patients participating in the mucosal explant study were symptomatic (≥2 nasal symptoms of AR) and were asked to mark the typical symptoms of AR on a visual analog scale (score, 0-10). The major symptom was selected based on the highest score on a visual analog scale.

Isolation and ALI cultures of primary NECs and transepithelial resistance measurement

Inferior turbinates were used for isolation of NECs. A highly purified NEC population was obtained, as reported previously.[20] The full methodology is provided in the Methods section in this article's Online Repository at www.jacionline.org.

Freshly isolated NECs were seeded on 0.4-μm, 0.33-cm^2 polyester Transwell inserts (Costar, Corning, NY) at a density of 10^5 cells per Transwell. Medium was refreshed every other day. Once NECs grew to complete confluence, the apical culture medium was removed to allow further cell differentiation in the ALI. At day 21 in the ALI, epithelial integrity was evaluated by using transepithelial resistance (TER) measurements with an EVOM/Endohm (WPI, Sarasota, Fla). Cultures not building up sufficiently (TER, <200 $\Omega \times cm^2$) were not included in experiments (14%). TER was measured in triplicate for each subject.

Paracellular flux measurements

Epithelial permeability as a surrogate marker of layer integrity was measured by using fluorescein isothiocyanate–dextran 4kDa (FD4; Sigma-Aldrich, St Louis, Mo). FD4 (2 mg/mL) was added apically to the ALI cultures at day 21, and the fluorescein isothiocyanate intensity of basolateral fluid was measured with a fluorescence reader (FLUOstar Omega; BMG Labtech, Ortenberg, Germany). FD4 concentration was calculated and is expressed in picomoles (pmols).

Cytokine stimulation experiments

NEC cultures from control subjects and patients with HDM-induced AR at the ALI were stimulated at day 21 by adding different cytokines to the basolateral compartment: 100 ng/mL IFN-γ or 10 ng/mL IL-4 (R&D Systems, Abingdon, United Kingdom). One hour before cytokine stimulation, 0.1 μmol/L FP (Sigma-Aldrich) or saline was added. TER and FD4 permeability was measured at 0, 24, 48, and 72 hours after stimulation. The optimal concentration for the different stimulation experiments was based on dose-response experiments in NEC cultures at the ALI.

Ussing chamber experiments for evaluation of mucosal explant integrity

Nasal biopsy specimens were mounted in Ussing chambers (Mussler Scientific Instruments, Aachen, Germany) with an opening of 0.017 cm^2 to evaluate mucosal integrity *ex vivo*, as described previously.[8] The methodology can be found in the Methods section in this article's Online Repository.