# Defective epithelial barrier in chronic rhinosinusitis: The regulation of tight junctions by IFN-γ and IL-4

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**Background: Chronic rhinosinusitis (CRS) is a common disease with still unclear pathophysiologic mechanisms. Epithelial tight junctions (TJs) have been shown to be involved in different chronic disorders, including bronchial asthma, inflammatory bowel diseases, and skin disorders. The regulation of epithelial barrier function and TJ expression has not been extensively studied in patients with CRS and in the paranasal sinus epithelium thus far.**

**Objective: We sought to elucidate the TJ expression pattern in the epithelium of the sinonasal mucosa and its regulation in patients with CRS.**

**Methods: Trans-tissue resistance was measured in biopsy specimens from healthy control subjects and patients with CRS with and without nasal polyps. TJ protein expression was determined by using immunofluorescence, Western blotting, and real-time PCR. Primary epithelial cell cultures from patients with CRS and control subjects were used in air-liquid interface (ALI) cultures for the measurement of transepithelial resistance (TER) and TJ expression. The effect of IFN-γ, IL-4, and IL-17 on ALI cultures was assessed.**

**Results: A decreased trans-tissue resistance was found in biopsy specimens from patients with CRS with nasal polyps along with an irregular, patchy, and decreased expression of the TJ molecules occludin and zonula occludens 1. TER was reduced in ALI cultures from patients with CRS with nasal polyps. The cytokines IFN-γ and IL-4 decreased TER, whereas IL-17 did not have any influence on epithelial integrity.**

**Conclusion: A defective epithelial barrier was found in patients with CRS with nasal polyps along with a decreased expression of TJ proteins. The disruption of epithelial integrity by IFN-γ and IL-4 *in vitro* indicates a possible role for these proinflammatory cytokines in the pathogenesis of patients with CRS. (J Allergy Clin Immunol 2012;130:1087-96.)**

***Key words:*** *Chronic rhinosinusitis, chronic sinusitis, tight junctions, occludin, claudin, zonula occludens, regulation, cytokines, leaky epithelium*

Chronic rhinosinusitis (CRS) is characterized by mucosal inflammation involving both the nasal cavity and paranasal sinuses, with potentially diverse causes.[1] It affects approximately 15% of the general population, leading to an immense effect on the quality of life of patients, as well as creating a large financial burden on health care systems worldwide.[2,3] According to the presence or absence of polyps in the sinonasal cavities, 2 clinical entities are distinguished. These subgroups not only reflect different clinical features but also show distinct immunologic patterns. In the Western population patients with chronic rhinosinusitis with nasal polyps (CRSwNP) have a $T_H2$-predominant type of inflammation,[4] whereas patients with chronic rhinosinusitis without nasal polyps (CRSsNP) display a $T_H1$ type.[5] Different disease-related processes have been identified in patients with CRS, yet its exact pathogenesis still remains unknown.

Tight junctions (TJs) consist of different transmembrane and scaffold adaptor proteins. They form the most apical intercellular junction between epithelial cells, providing an apicobasolateral differentiation pattern. On the one hand, they are responsible for the regulation of paracellular flux and epithelial impermeability. In addition, they also prevent foreign particles, such as allergens, from entering the subepithelial layers. On the other hand, an opening of TJs can lead to drainage of inflammatory cells toward the lumen, supporting the resolution of phlogistic processes. Consequently, they can be considered gatekeepers that could contribute to both aggravation of inflammation-related tissue damage or resolution of inflammation through drainage. Different members of the TJ proteins have been identified, including occludin, tricellulin, the family of claudins, and junctional adhesion molecules.[6] They form intercellular homodimers/heterodimers between neighboring cells. On the cytoplasmic side, they bind to the actin cytoskeleton through associated proteins, such as the zonula occludens (ZO) family and cingulin.

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*Abbreviations used*

ALI: Air-liquid interface
CRS: Chronic rhinosinusitis
CRSsNP: Chronic rhinosinusitis without nasal polyps
CRSwNP: Chronic rhinosinusitis with nasal polyps
ECP: Eosinophil cationic protein
FITC: Fluorescein isothiocyanate
HSEC: Human primary sinonasal epithelial cell
MMP: Matrix metalloproteinase
TER: Transepithelial resistance
TJ: Tight junction
ZO: Zonula occludens

Disturbed TJs can lead to the entrance of pathogens and environmental antigens, including allergens, into the organism. Multiple disorders have been linked to defective or altered TJs, such as seen in patients with inflammatory intestinal diseases,[7] including Crohn disease; skin diseases, such as atopic dermatitis[8] and psoriasis[9]; and bronchial asthma.[10] Very recently, a disrupted TJ layer in biopsy specimens and increased permeability *in vitro* were found in asthmatic patients.[11]

However, changes in TJ arrangement in the nasal cavity, a region heavily exposed to environmental antigens, are not understood in the context of chronic inflammation. A defective barrier function has been suggested in patients with CRS.[12] TJs have not been studied extensively in the context of the nose and paranasal sinuses thus far. It is known that in patients with acute rhinitis involving rhinovirus, transepithelial resistance (TER) is decreased and ZO-1 is disrupted.[13] ZO-1 was also shown to be downregulated in nasal polyposis along with epithelial dedifferentiation.[14] Weakened desmosomal junctions were present in patients with CRSwNP.[15] However, a clear comparison of the 2 disease entities with regard to TJs has been lacking.

This study aims to investigate TJ function, expression, and regulation in patients with CRSwNP and those with CRSsNP. We demonstrate that leaky epithelium is present *in vivo* and *in vitro* in patients with CRSwNP along with a downregulation of claudin-4 and occludin mRNA in biopsy specimens from patients with CRSwNP. In view of the inflammatory processes in patients with CRS, the regulation by cytokines was assessed.[16] TER of air-liquid interface (ALI) cultures was decreased by the $T_H1$ cytokine IFN-γ and the $T_H2$ cytokine IL-4, whereas the $T_H17$ cytokine IL-17A had no effect.

## METHODS

### Patients

Patients undergoing paranasal sinus surgery because of CRS with and without nasal polyposis were enrolled as study patients. Patients undergoing paranasal sinus surgery for noninflammatory reasons (ie, cerebrospinal fluid leak, bullous middle turbinate, and those undergoing septal surgery) were used as healthy control subjects. Nasal or systemic corticosteroid administration up to 4 weeks before surgery was considered an exclusion criterion. Patients with CRS caused by underlying systemic disorders were not included. Total serum IgE and specific IgE levels to common aeroallergens were obtained, when feasible. Patients were considered allergic if total IgE levels exceeded 100 kU/L or the allergen-specific IgE level was greater than 3.51 kU/L in addition to the patient's history being suggestive for allergies (see Table E1 in this article's Online Repository at www.jacionline.org). The study protocol was approved by the ethics committee and review board of the canton Zurich and was conducted according to the latest version of the Declaration of Helsinki.

Biopsy specimens were taken during paranasal sinus or skull base surgeries and septo/-septorhinoplasties after achievement of general anesthesia. In patients with CRSwNP, polypoid tissue was used for all analyses, whereas in patients with CRSsNP, biopsy specimens were obtained from the affected maxillary/ethmoidal or sphenoidal mucosae. Biopsy specimens in control subjects were obtained from different locations, including the inferior/middle turbinates, uncinate process, and paranasal sinuses, to minimize the effects of a potential bias caused by the site of tissue origin. In a subgroup of patients, we decided to only collect the surface layers of the tissue by means of curettage and scraping.

### Ussing chamber and trans-tissue resistance

Tissue samples with a diameter of approximately 7 mm were transported in 0.9% NaCl on ice. They were placed to cover the 4-mm-wide opening of a CHM8 Ussing chamber (World Precision Instruments, Berlin, Germany). Two percent Agarose (Gibco-BRL, Invitrogen, Basel, Switzerland) in 150 mmol/L KCl (Fluka, Sigma-Aldrich, St Louis, Mo) was used to fill the electrodes, and PBS (Gibco-BRL, Invitrogen) was used in the chamber bath. Measurements were obtained in $\Omega \times cm^2$ by using a Millicell-ERS volt ohm meter (Millipore, Temecula, Calif).

### Human primary sinonasal epithelial cell lines

Tissue samples were cut into pieces of approximately 1 to 2 mm and trypsinized for 3 hours at 37°C (5% $CO_2$; Trypsin EDTA 0.05%, Invitrogen). Trypsin was neutralized with TNS (Lonza, Basel, Switzerland), and the tissue was passed through a 70-μm nylon mesh. The obtained cells were seeded in 75-$cm^2$ plastic culture flasks and cultured in bronchial epithelial growth medium including the Single Quot Bullet Kit (Lonza). Medium was changed after 24 hours and every second day from then on. Cells were harvested at a confluence of 90% by using trypsinization.

### Cell purity

Cell purity was determined by using vimentin/cytokeratin staining for all human primary sinonasal epithelial cells (HSECs) that were isolated in this study (see Fig E1 in this article's Online Repository at www.jacionline.org). The full methodology is provided in the Methods section in this article's Online Repository at www.jacionline.org.

### ALI cultures, cytokine stimulations, and TER

HSECs obtained from healthy subjects and patients with CRS in passages 2 or 3 were seeded onto 6.5-mm-diameter polyester membranes with a pore size of 0.4 μm (Costar; Corning, Corning, NY) at a density of 110,000 cells per well. Dulbecco modified Eagle medium (Gibco-BRL, Invitrogen) with fresh retinoic acid was mixed 1:1 with bronchial epithelium basal medium (BEBM, Lonza) supplemented with the Single Quot kit except for triiodothyronine and retinoic acid (Lonza) and used as the medium in ALI cultures. Experiments were conducted with cells from different donors. Each donor culture was measured in a minimum of duplicates in multiple well systems to minimize variation within 1 experiment, and average numbers were used for analyses. Cell cultures were performed with primary cell lines from comparable passage numbers. Passage 2 was used in 16 experiments, and third-passage HSECs were used in 4 different cultures (2 control subjects and 2 patients with CRSwNP, respectively). Medium was changed every second day. Once the cells grew to complete confluence, the apical compartment was freed of any medium to allow further cell differentiation at the ALI to take place. TER was measured daily in $\Omega \times cm^2$ by using a Millicell ERS Volt-Ohm Meter (Millipore). The highest measurement in the time course was used for comparison between different cell lines. Wells not building up sufficient TER (<200 $\Omega \times cm^2$) were not used in experiments. Hematoxylin and eosin staining of ALI culture cross-sections can be seen in Fig E2 in this article's Online Repository at www.jacionline.org.

Stimulation experiments were initiated 2 days after reaching maximal TER. All ALI cultures from control subjects, patients with CRSwNP, and patients with CRSsNP were used for experiments. The abovementioned