

Oncostatin M promotes mucosal epithelial barrier dysfunction, and its expression is increased in patients with eosinophilic mucosal disease

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Background: Epithelial barrier dysfunction is thought to play a role in many mucosal diseases, including asthma, chronic rhinosinusitis (CRS), and eosinophilic esophagitis.

Objective: The objective of this study was to investigate the role of oncostatin M (OSM) in epithelial barrier dysfunction in human mucosal disease.

Methods: OSM expression was measured in tissue extracts, nasal secretions, and bronchoalveolar lavage fluid. The effects of OSM stimulation on barrier function of normal human bronchial epithelial cells and nasal epithelial cells cultured at the air-liquid interface were assessed by using transepithelial electrical resistance and fluorescein isothiocyanate–dextran flux. Dual-color immunofluorescence was used to evaluate the integrity of tight junction structures in cultured epithelial cells. **Results:** Analysis of samples from patients with CRS showed that OSM mRNA and protein levels were highly increased in

nasal polyps compared with those seen in control uncinat tissue ($P < .05$). OSM levels were also increased in bronchoalveolar lavage fluid of allergic asthmatic patients after segmental allergen challenge and in esophageal biopsy specimens from patients with eosinophilic esophagitis. OSM stimulation of air-liquid interface cultures resulted in reduced barrier function, as measured by decreased transepithelial electrical resistance and increased fluorescein isothiocyanate–dextran flux ($P < .05$). Alterations in barrier function by OSM were reversible, and the viability of epithelial cells was unaffected. OSM levels in lysates of nasal polyps and uncinat tissue positively correlated with levels of α_2 -macroglobulin, a marker of epithelial leak, in localized nasal secretions ($r = 0.4855$, $P < .05$).

Conclusions: These results suggest that OSM might play a role in epithelial barrier dysfunction in patients with CRS and other mucosal diseases. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: Oncostatin M, epithelial barrier, chronic rhinosinusitis, eosinophilic esophagitis, atopic asthma, transepithelial electrical resistance, tight junctions

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Mucosal epithelium maintains tissue defense and homeostasis under normal circumstances through many functions, including mucociliary action, production of mucus and antimicrobial peptides, maintenance of air-surface liquid, and ion transport.¹ The epithelial barrier also serves as a first line of defense by creating a physical barrier to pathogens, allergens, and other environmental factors. The integrity of the epithelial barrier is largely determined by the structure of intercellular epithelial junctions, including tight junctions, adherens junctions, and desmosomes.² Upon breach of the epithelial barrier, pathogens, allergens, and environmental factors can synergize with alarmins and danger-associated molecular patterns, which are released in response to cellular damage, thereby activating innate and adaptive immune responses.³ The ability of the immune system to neutralize the pathogenic stimulus and repair tissue damage is critical for restoration of the tissue to a normal homeostatic state.

Dysregulation of epithelial maintenance and repair of barrier function has been implicated in the pathogenesis of many diseases, including asthma, atopic dermatitis, eosinophilic esophagitis (EoE), and chronic rhinosinusitis (CRS). The airway epithelium of allergic asthmatic patients has been shown to have a number of defects, including decreased expression of intercellular junction proteins, increased susceptibility to injury, dysregulated repair, and increased proliferation.⁴⁻⁷ Dysregulated epithelial barrier function, as shown through abnormal electrical

Abbreviations used

ALI:	Air-liquid interface
BAL:	Bronchoalveolar lavage
CRS:	Chronic rhinosinusitis
CRSsNP:	Nonpolypoid chronic rhinosinusitis
CRSwNP:	Polypoid chronic rhinosinusitis
EoE:	Eosinophilic esophagitis
FITC:	Fluorescein isothiocyanate
JAK:	Janus kinase
NEC:	Nasal epithelial cell
NHBE:	Normal human bronchial epithelial cell
OSM:	Oncostatin M
OSMR:	Oncostatin M receptor β chain
RA:	Rheumatoid arthritis
STAT:	Signal transducer and activator of transcription
TEER:	Trans epithelial electrical resistance
UT:	Uncinate tissue

and ion permeability, has also been demonstrated in patients with CRS.⁸⁻¹¹ Furthermore, sinus epithelium in nasal polyps of patients with CRS was shown to have morphologic changes consistent with epithelial dysfunction, including basal cell proliferation and goblet cell hyperplasia, in addition to increased epithelial damage and decreased tissue resistance compared with control epithelium.^{8,12-14}

Oncostatin M (OSM) is a member of the IL-6 family of cytokines and has been shown to signal through 2 receptors; the type 1 receptor is a heterodimer of leukemia inhibitory factor receptor and gp130, and the type 2 receptor is a heterodimer of OSM receptor β chain (OSMR) and gp130. OSM has been shown to be expressed by multiple cell types of hematopoietic lineage but is not known to be expressed by epithelium, fibroblasts, or smooth muscle, the cells that express the different forms of the OSM receptor.¹⁵ Previous studies have reported increased OSM levels in sinus tissue from patients with allergic rhinitis, psoriatic skin, and sputum of asthmatic patients with irreversible airflow obstruction.¹⁶⁻¹⁸ Moreover, intratracheal administration of adenoviral OSM to mice was shown to be sufficient to induce a type 2 immune response in the lung that was characterized by goblet cell hyperplasia; formation of bronchial-associated lymphoid tissue; eosinophil infiltration; production of IL-4, IL-5, IL-13, and eotaxins; and airway hyperresponsiveness in a mouse model.^{19,20} Studies with reconstituted epidermis have shown that OSM treatment decreased expression of filaggrin, a molecule that is important for barrier function in keratinocytes, suggesting that OSM could contribute to barrier dysfunction in the skin.²¹ OSM has also been shown to decrease barrier function in endothelial cells.²²

Given that OSM is sufficient to induce a type 2 immune response in the lung¹⁹ and has been shown to potentially contribute to barrier dysfunction in the endothelium and skin, we tested whether levels of OSM were increased in patients with CRS and contributed to epithelial barrier dysfunction in patients with this disease.

METHODS**Patients and tissue sample collection**

Control subjects and patients with CRS were recruited from the Allergy-Immunology and Otolaryngology clinics of the Northwestern

Medical Faculty Foundation and the Northwestern Sinus Center at Northwestern Medicine. Uncinate tissue (UT), nasal polyps, epithelial scrapings from the inferior turbinate, nasal lavage fluid, and sponge-based nasal secretions were obtained during routine functional endoscopic sinus surgery from patients with CRS, as defined by the American Academy of Otolaryngology–Head and Neck Surgery Chronic Rhinosinusitis Task Force.²³ Patients with established immunodeficiency, pregnancy, a coagulation disorder, classic allergic fungal sinusitis, or cystic fibrosis were excluded from this study. Control subjects did not have a history of sinonasal inflammation, and tissue was collected during endoscopic skull-base tumor excisions, intranasal procedures for obstructive sleep apnea, and facial fracture repairs. The characteristics of patients donating sinus samples are shown in Table E1 in this article's [Online Repository](http://www.jacionline.org) at www.jacionline.org. Nasal secretions from the middle meatus were collected by using an endoscopically placed 0.375-inch polyvinyl alcohol sponge (Medtronic, Minneapolis, Minn) that was inserted between the middle turbinate and the adjacent uncinate process for 10 minutes before removal. The polyvinyl alcohol sponges were then centrifuged at 14,000 rpm for 10 minutes and extracted with a further 100 μ L of PBS and 1% protease inhibitor cocktail to collect nasal secretions. Segmental allergen challenge and bronchoalveolar lavage (BAL) were performed, as previously described.^{24,25} The patient cohort used for segmental allergen challenge was previously described.²⁴ Biopsy specimens from control subjects and patients with EoE were obtained by means of endoscopic collection on the initial visit, as previously described, and the characteristics of these patients are shown in Table E2 in this article's [Online Repository](http://www.jacionline.org) at www.jacionline.org.²⁶ The diagnosis of EoE was based on an eosinophil count of 15 or more per high-power field in an esophageal biopsy section.

Quantitative RT-PCR and microarray analysis

RNA isolated from tissue samples was prepared, as previously described.²⁷ Single-strand cDNA was synthesized from 0.5 μ g of total RNA with SuperScript II reverse transcriptase and random primers (Invitrogen, Carlsbad, Calif). Semiquantitative real-time RT-PCR was performed with an Applied Biosystems 7500 Sequence Detection System (Applied Biosystems, Foster City, Calif) in 20- μ L reactions (10 μ L of 2 $\times$ TaqMan Master mix [Applied Biosystems], 400 nmol/L of each primer, and 200 nmol/L of TaqMan probe plus 10 μ g of cDNA). Primer and probe sets for OSM (Hs00968300_g1), OSMR (Hs00384276.m1), and β -glucuronidase (4326320E) were purchased from Applied Biosystems. Several housekeeping genes were screened for expression in UT and nasal polyps; β -glucuronidase was found to have the most consistent expression between these 2 tissue types and was used as a housekeeping gene when analyzing gene expression in both UT and nasal polyps. Primers (forward: CTGGCCGGACCTGACT, reverse: GCAGCCGTGGCCATCTC) and probe (6-VIC-CACCAACACGCCGGA-MGB) for β -actin were synthesized by IDT technologies (Coralville, Iowa) and used to detect the housekeeping gene in EoE studies. Microarray analysis was performed, as previously described.²⁸

Protein detection

OSM protein was detected in tissue and nasal secretions by using a bead array assay for OSM, according to the manufacturer's instructions (EMD Millipore, Billerica, Mass). Sinus tissue extracts were prepared, as previously described.²⁹ OSM levels were analyzed on a Luminex 200 instrument (Life Technologies, Grand Island, NY). α_2 -Macroglobulin protein was detected in nasal secretions by using an ELISA, according to the manufacturer's instructions (Alpco, Salem, NH). Albumin and OSM protein were detected in BAL fluid after segmental antigen challenge, as previously described.²⁴

Cell culture and barrier assessment

Normal human bronchial epithelial cells (NHBEs; Lonza, Walkersville, Md) and nasal epithelial cells (NECs) from our clinical repository were seeded onto transwells and grown to confluence in bronchial epithelial growth medium (Lonza). Once confluent, medium from the upper chamber of the transwell was removed, constituting the transition to air-liquid interface (ALI)

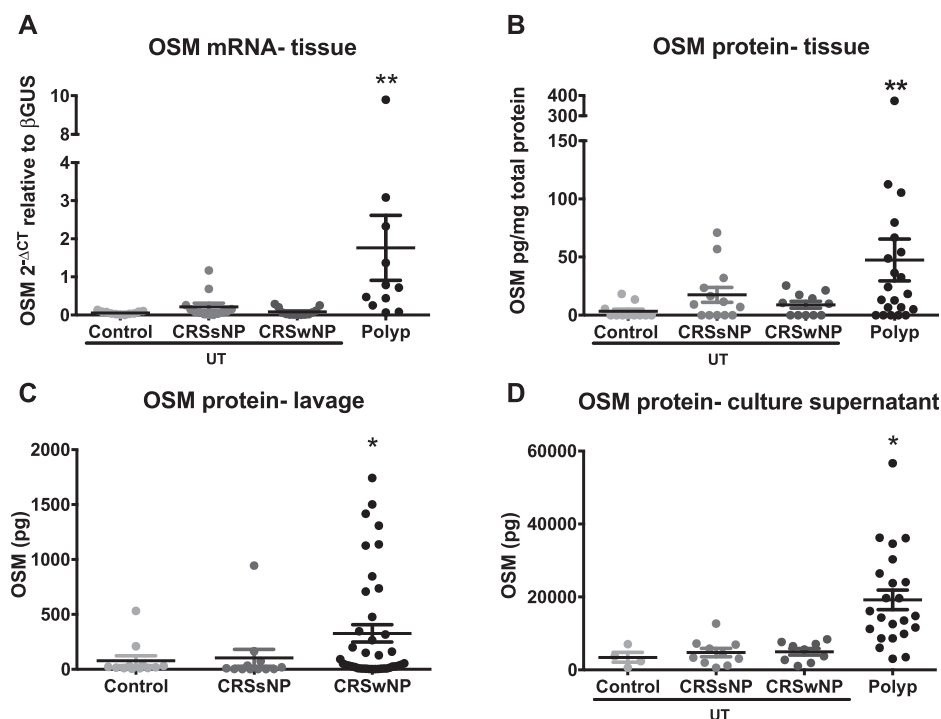


FIG 1. OSM levels were increased in patients with CRS. **A**, OSM mRNA levels were increased in nasal polyps compared with those in UT from control subjects, patients with CRSsNP, and patients with CRSwNP (1.76 ± 0.85 vs 0.06 ± 0.013 , respectively; $n = 11-13$; $P < .01$, Kruskal-Wallis test). **B**, OSM levels were increased in nasal polyp tissue compared with those in control UT, as measured by using Luminex (3.38 ± 1.95 vs 47.49 ± 17.98 pg/mg, respectively; $n = 11-19$; $P < .01$, Kruskal-Wallis test). **C**, OSM protein levels were increased in nasal lavage fluid of patients with CRSwNP patients compared with those in patients with CRSsNP (78.50 ± 44.16 vs 78.83 ± 78.83 pg, respectively; $n = 10-40$; Kruskal-Wallis test). **D**, OSM protein levels were increased in culture supernatants of nasal polyp tissue compared with those in UT from patients with CRSsNP and those with CRSwNP (19.1 ± 2.6 vs 4.7 ± 1.1 and 4.9 ± 0.89 ng, respectively; $n = 4-23$; Kruskal-Wallis test). * $P < .05$ and ** $P < .01$.

culture conditions. NHBEs and NECs were fully differentiated at day 21 of ALI culture. Barrier function was measured by using 2 methods: transepithelial electrical resistance (TEER) and fluorescein isothiocyanate (FITC)-dextran flux. Fully differentiated NHBEs and NECs were stimulated with recombinant human OSM (R&D systems, Minneapolis, Minn) at 100 ng/mL for 48 hours, unless otherwise noted. TEER was measured with a voltammeter (World Precision Instruments, Sarasota, Fla). Dextran flux was measured by placing 10 kDa FITC-conjugated dextran (Sigma, St Louis, Mo) in the upper chamber of the transwell for 30 minutes to assess barrier function, after which supernatants were harvested from the lower chamber, and a Molecular Devices Spectramax Gemini EM fluorimeter (Molecular Devices, Sunnyvale, Calif) was used to measure the amount of FITC-dextran that crossed through the cell layer. Cytotoxicity of OSM treatment was measured with a Cytotoxicity Detection Kit Plus (Roche Applied Science, Indianapolis, Ind). Four-day *ex vivo* culture of UT and nasal polyps was performed, as previously described.²⁸

Immunofluorescence staining

NHBE and NEC monolayers grown on transwell inserts were fixed in 10% formalin before washing with PBS. The cell layers were blocked with PBS containing 5% goat serum (Vector Laboratories, Burlingame, Calif), 1% BSA (Sigma), and 1.6% Triton X-100 (Sigma) for 1 hour at room temperature and then incubated with rabbit anti-occludin for 1 hour at room temperature (1:200, 71-1500; Life Technologies). The membranes were then incubated with secondary antibody for 1 hour at room temperature with Alexa Fluor 488 goat anti-rabbit antibody (Life Technologies). Slides were mounted with SlowFade Gold Antifade Reagent with DAPI counterstain (Life

Technologies). Imaging was performed with a Nikon A1R confocal microscope using the 40× objective. Images were processed with ImageJ software (National Institutes of Health, Bethesda, Md).

Statistical analysis

All data were analyzed with GraphPad Prism 6 software (GraphPad Software, La Jolla, Calif) and reported as means $\pm$ SEMs. Differences between groups were analyzed by using a nonparametric Kruskal-Wallis ANOVA or Mann-Whitney *U* test. Correlations were assessed by using the Pearson correlation.

RESULTS

OSM levels were increased in patients with CRS

We first wanted to determine whether OSM expression was increased in polyps from patients with CRSwNP. Using data from a previously published microarray study (GEO data set GSE36830),³⁰ we found that OSM expression was increased in nasal polyps of patients with CRS compared with those seen in UT from control subjects (Fig E1, A, in this article's [Online Repository](http://www.jacionline.org) at www.jacionline.org). We then went on to verify this finding at the mRNA and protein levels by using a separate set of samples. Consistent with our array results, we found that OSM mRNA levels in nasal polyps were increased compared with those in UT from control subjects (Fig 1, A). In addition,

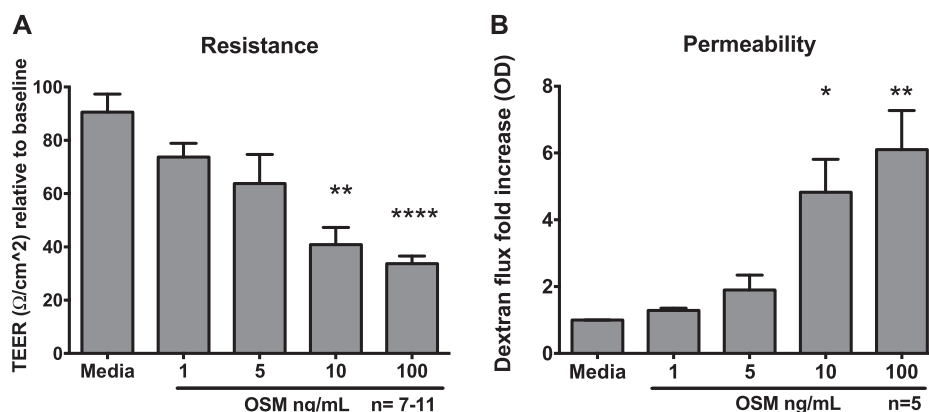


FIG 2. OSM decreased barrier function in airway epithelium. ALI cultures of NHBEs were grown until fully differentiated at day 21 of culture, and then the cells were left unstimulated or stimulated with OSM at the specified concentration. **A**, OSM stimulation resulted in a concentration-dependent decrease of TEER. Data are presented as the change in resistance over the 48-hour stimulation normalized to resistance at 0 hours to show the percentage of resistance lost during stimulation: (48-h TEER/0-h TEER) * 100 (n = 7-11; $P < .0001$, Kruskal-Wallis test). **B**, Dextran flux across the cell layer was increased with OSM stimulation, suggesting decreased barrier function after OSM stimulation (n = 5; $P < .001$, Kruskal-Wallis test). Data are means $\pm$ SEMs. * $P < .05$, ** $P < .01$, and **** $P < .0001$.

OSM protein levels were increased in nasal polyps compared with those in control UT and in nasal lavage fluid from patients with polypoid chronic rhinosinusitis (CRSwNP) compared with patients with nonpolypoid chronic rhinosinusitis (CRSsNP; Fig 1, B and C). Analysis of supernatants from an *ex vivo* 4-day culture of sinonasal tissues also showed increased OSM levels in the supernatants of cultures of nasal polyp tissues compared with those in UT from patients with CRSsNP and those with CRSwNP (Fig 1, D).²⁸ To test whether the tissue might contain OSM-responsive cells, we evaluated expression of OSMR and found that OSMR mRNA levels were also increased in nasal polyps compared with those in control UT (see Fig E1, B).

OSM decreased barrier function in cultured airway epithelium

To study the effect of OSM stimulation on airway epithelium, we used an ALI culture system, which allowed us to study differentiated epithelium *in vitro*. Fully differentiated NHBEs were left unstimulated or stimulated with various concentrations of OSM (1-100 ng/mL for 48 hours). Barrier function of the NHBE cultures was assessed by using TEER, which measures the resistance of the cell layer, and paracellular dextran flux. In an intact barrier TEER will be high, whereas dextran flux will be low. OSM stimulation of NHBEs induced a concentration-dependent decrease in TEER (Fig 2, A) and an increase in dextran flux (Fig 2, B), which are both characteristic of significant loss of barrier integrity. ALI monolayer cultures of NECs also showed decreased TEER (see Fig E2, A, in this article's [Online Repository at www.jacionline.org](http://www.jacionline.org)) and increased dextran flux (see Fig E2, B) when stimulated with 100 ng/mL OSM. Importantly, OSM stimulation of NHBEs did not decrease cell viability, as assessed by means of trypan blue staining (data not shown). In addition, there was no difference in lactate dehydrogenase levels in the supernatants of OSM-treated cells when compared with those in control cells (see Fig E2, D). NEC cultures were stimulated with a high concentration of OSM for 2 days (100 ng/mL) and then washed and allowed to recover for 7 days in ALI culture

medium to determine whether the effects of OSM treatment on epithelial barrier function were reversible. We found no difference between TEER measurements after the 7-day recovery period and TEER measured before OSM stimulation (baseline), indicating that the effect of OSM on barrier function was reversible (see Fig E2, C).

OSM stimulation altered organization of intercellular junctions

Intercellular junctions, particularly tight junctions, are largely responsible for barrier function of the mucosal epithelium. To determine whether OSM stimulation altered expression of key junctional proteins, we analyzed mRNA expression of occludin, claudin 1, E-cadherin, and zonula occludens 1. We found that OSM stimulation of NHBEs had no effect on the level of gene expression of these molecules (data not shown). Additionally, OSM stimulation did not change occludin protein expression, as measured by using Western blotting (data not shown). We next determined whether OSM disrupted localization of junctional proteins. Using immunofluorescence analysis of occludin, we found the characteristic lattice-like structure of occludin-positive tight junctions between epithelial cells of both unstimulated NHBE and NEC cultures (Fig 3). However, after OSM stimulation at 100 ng/mL in both NHBEs and NECs, it was clear that the organization of the tight junction had been lost (Fig 3), indicating that OSM could disrupt tight junction localization, even though it did not affect expression of tight junction proteins. Additionally, when NHBEs were stimulated with various concentrations of OSM, tight junction structure was also lost at 10 ng/mL OSM, whereas it was intact at 1 ng/mL (Fig 3).

OSM was associated with impaired barrier function in patients with CRS

To determine whether there was an epithelial cell-intrinsic defect in patients with CRS, we next assessed whether NECs from patients with CRS had decreased barrier function compared with

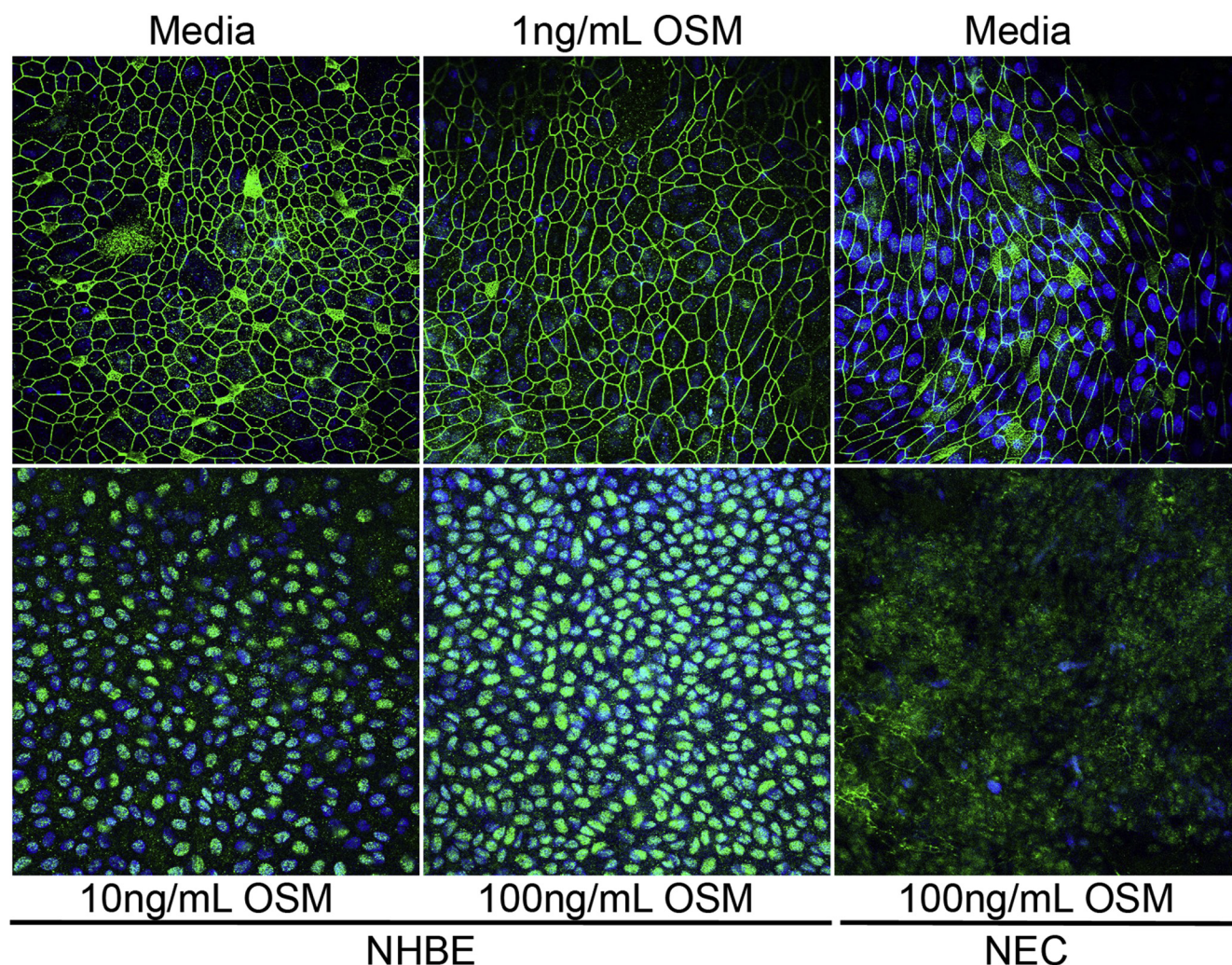


FIG 3. OSM disrupted organization of tight junctions. NHBEs were left unstimulated or stimulated with indicated concentrations of OSM for 48 hours. NECs were either left unstimulated or stimulated with 100 ng/mL OSM. The tight junction protein occludin is stained in green, and cell nuclei are stained in blue (NHBEs, $n = 3$; NECs, $n = 3$).

that seen in control subjects. NECs collected *in vivo* from the inferior turbinate from patients with CRSsNP, patients with CRSwNP, and control subjects were cultured *ex vivo* at the ALI, and TEER was measured weekly. NECs from each group had robust barrier function over the course of the assay, and no differences were seen among the groups (Fig 4, A). This suggests that the cause of barrier dysfunction in patients with CRS is probably extrinsic to the epithelium. Because OSM levels were increased in patients with CRS and significantly decreased epithelial barrier function *in vitro*, we next wanted to determine whether OSM might contribute to epithelial barrier dysfunction *in vivo*. We collected nasal secretions from specific locations within the middle meatus using a sponge in the sinus cavity placed adjacent to the polyp tissue and measured α_2 -macroglobulin levels, which have been used as a macromolecular marker of epithelial leak, in nasal secretions collected from the sponges.^{31,32} OSM levels in tissue lysates from patients with CRSsNP and those with CRSwNP were significantly positively correlated with α_2 -macroglobulin levels in adjacent nasal secretions ($P < .05$, $r = 0.4855$; Fig 4, B), suggesting that

there was a relationship between OSM levels and epithelial barrier dysfunction *in vivo*.

OSM levels were increased in patients with EoE and those with atopic asthma

We next wanted to determine whether OSM levels were increased in patients with other mucosal disorders with dysregulated type 2 immune responses and barrier dysfunction. First, we analyzed mRNA from endoscopic esophageal biopsy specimens from control subjects and patients with EoE. We found that OSM expression was increased in biopsy specimens from patients with EoE compared with those from control subjects (Fig 5, A). We next analyzed OSM protein levels in the BAL fluid of allergic asthmatic patients after segmental antigen challenge.^{24,25} Although OSM was not present in BAL fluid of allergic asthmatic patients when challenged with saline, OSM levels were significantly increased by 18 to 20 hours after allergen challenge (Fig 5, B). Additionally, we measured levels of human serum

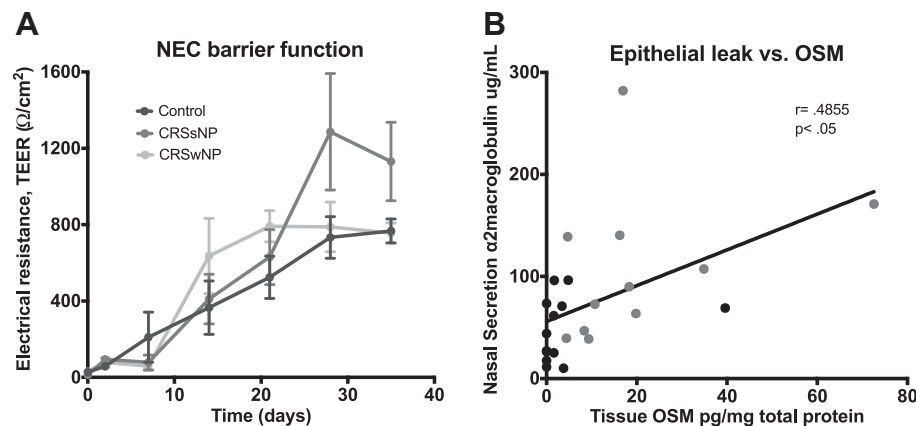


FIG 4. OSM expression correlated with a marker of epithelial leak *in vivo*. **A**, ALI cultures of NECs from inferior turbinate from control subjects, patients with CRSsNP, and patients with CRSwNP show no difference in TEER ($n = 7-11$). Data are means $\pm$ SEMs. **B**, OSM levels in nasal polyps and UT from patients with CRSsNP correlated with epithelial leak, as determined by assessment of $\alpha 2$ -macroglobulin levels in adjacent nasal secretions (Pearson $r = 0.4855$, $n = 11-13$, $P < .05$). Gray dots signify nasal polyp tissue, and black dots signify UT of patients with CRSsNP.

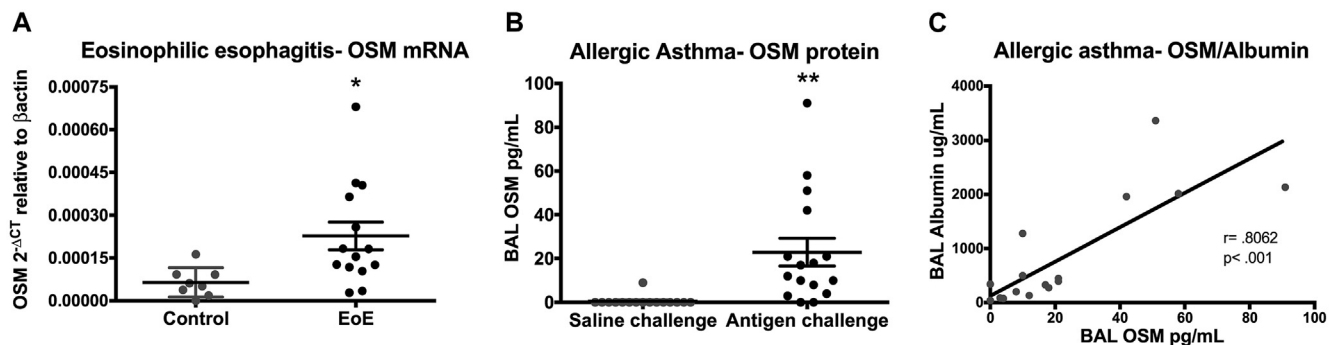


FIG 5. Levels of OSM were increased in patients with EoE and upon allergen challenge in allergic asthmatic patients. **A**, OSM mRNA levels were increased in esophageal biopsy specimens of patients with EoE compared with those in control subjects ($6.4\text{e-}005 \pm 1.8\text{e-}005$ vs $0.00024 \pm 4.9\text{e-}005$, respectively; $n = 8-13$; $P < .01$, Mann-Whitney U test). **B**, OSM protein levels were increased in BAL fluid of allergic asthmatic patients after segmental allergen challenge compared with that in BAL fluid of patients challenged with saline (0.56 ± 0.56 vs 22.8 ± 6.3 , respectively; $n = 16$; $P < .0001$, Mann-Whitney U test). **C**, OSM levels in BAL fluid after allergen challenge in allergic asthmatic patients correlate with levels of human serum albumin (Pearson $r = 0.8062$, $P < .001$). * $P < .05$ and ** $P < .01$.

albumin, another macromolecular marker associated with epithelial leak, in the BAL fluid of these patients, and we found that human serum albumin levels correlated positively with OSM levels in BAL fluid ($P < .001$, $r = 0.8062$; Fig 5, C). Collectively, these data indicate that OSM levels were associated with barrier dysfunction in patients with other mucosal diseases mediated by type 2 inflammation.

DISCUSSION

We have found that OSM levels were increased in nasal polyp tissues derived from patients with CRSwNP (Fig 1). Furthermore, studies with cultured bronchial and nasal epithelium demonstrated that stimulation with OSM at concentrations similar to those seen *in vivo* were sufficient to cause significant loss of barrier function and disorganization of tight junction structure (Figs 2 and 3). Additionally, OSM expression detected in nasal polyp tissue lysates correlated with levels of $\alpha 2$ -macroglobulin, a marker

of epithelial leak in localized nasal secretions (Fig 4). Together, these data suggest that OSM might be mediating epithelial barrier dysfunction in patients with CRS, potentially leading to a chronic inflammatory state through increased exposure of the tissue to environmental factors, including pathogens, allergens, and pollutants (Fig 6). In support of this hypothesis, we found increased OSM mRNA levels in esophageal biopsy specimens from patients with EoE compared with those from control subjects (Fig 5) and increased OSM protein levels after allergen challenge in allergic asthmatic patients that correlated with epithelial leak in the BAL fluid (Fig 5). Although these findings suggest that OSM might play a role in other diseases of mucosal epithelium that are also associated with type 2 inflammation, further studies are needed to verify these findings in patients with other diseases.

The repair of epithelial damage occurs in 2 basic steps. First, basal cells proliferate and migrate into the wound to cover the damaged area, and second, progenitor cells stop proliferating and

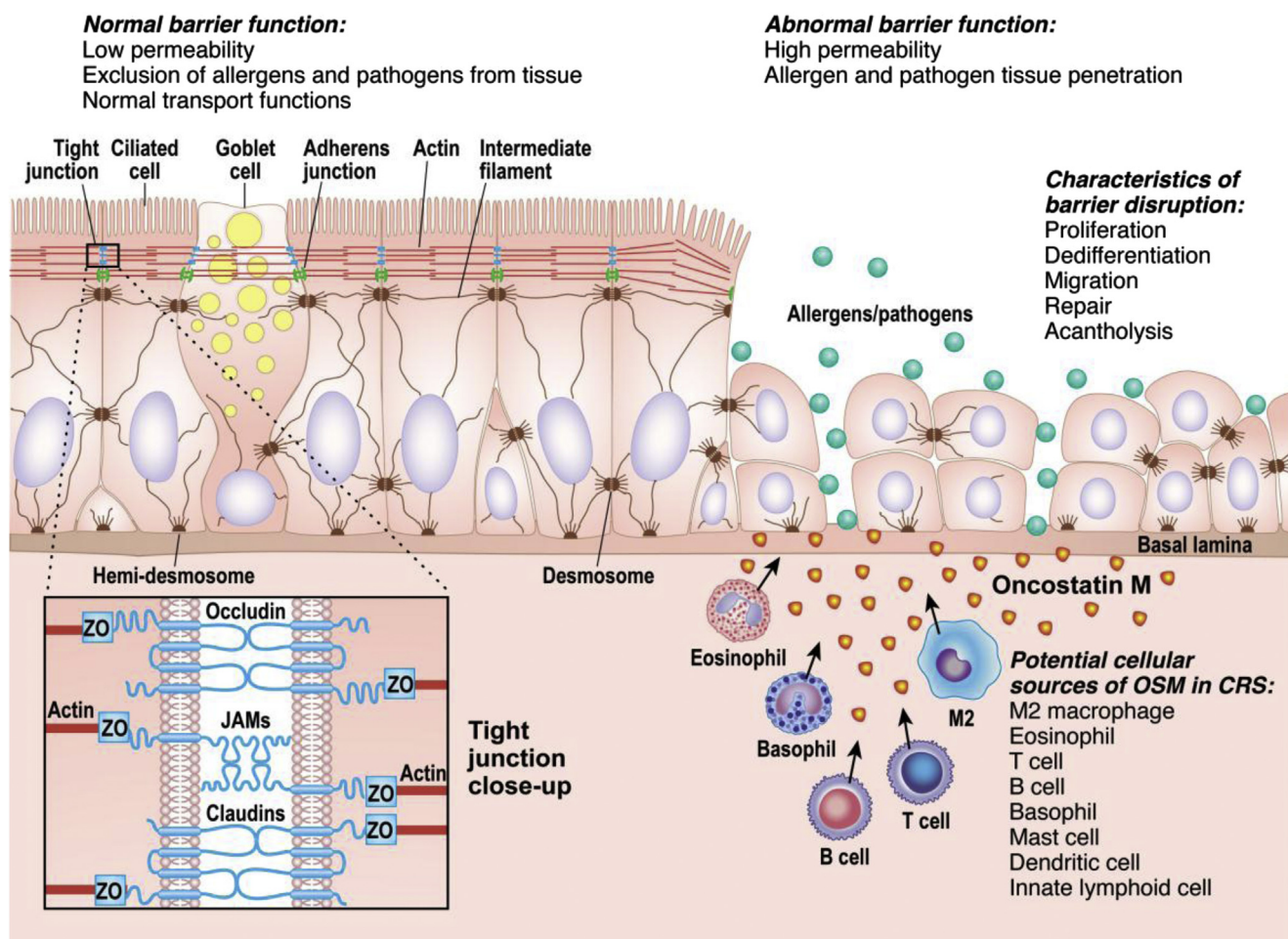


FIG 6. Proposed model for the role of OSM in epithelial barrier dysfunction in patients with CRS. Airway epithelium develops barrier function through a complex network of intercellular junctions that adhere the cells to each other and the cytoskeleton, as illustrated on the left side of the figure. Barrier dysfunction, as illustrated on the right side of the figure, can occur after exposure to OSM and leads to a chronic inflammatory state caused by the presence of environmental factors that would not otherwise have access to the tissue. *JAMs*, Junctional adhesion molecules; *ZO*, zonula occludens.

differentiate into ciliated or goblet cells, and intercellular junctions are then formed to re-establish barrier function in the repaired tissue.³³ Although the mechanism of OSM-mediated barrier dysfunction in mucosal epithelium *in vitro* is currently unknown, increased OSM levels might be one signal that initiates the first step of repair after cellular damage. However, *in vivo* in a diseased state, high OSM levels, or the absence of a signal to initiate the second step of the repair process, could potentially prevent induction of the second step, leading to a chronically proliferative state in which differentiation and establishment of barrier function does not occur. OSM might achieve its barrier-disrupting effects *in vitro* through a number of signaling pathways, including signal transducer and activator of transcription (STAT) 1, STAT3, STAT5, phosphatidylinositol 3-kinase, and the mitogen-activated protein kinase pathway.^{34–38} Future studies will be aimed at determining which of these pathways is responsible for the *in vitro* effects of OSM. Whether OSM disrupts barrier function *in vivo* and the molecular pathway through which it might do so will be challenging to assess. The STAT3 pathway is of particular interest because it mediates both host defense

responses and barrier maintenance and repair. *In vivo* studies of STAT3 phosphorylation in fresh primary epithelial cells from either healthy subjects or patients with CRS have proved to be technically difficult. Although we have previously shown a decrease in STAT3 phosphorylation in whole nasal polyp tissue, it is quite possible that the level of STAT3 activation in epithelial cells differs from that of the whole polyp tissue.^{39,40} The results of *in vitro* studies of possible signaling pathways for OSM's effects will hopefully point the way toward the pathways that should be the focus of future *in vivo* studies.

A recent study by Soyka et al¹³ investigated whether *ex vivo* sinus tissue from patients with CRS had decreased resistance compared with control tissue. Large sinus tissue biopsy specimens were obtained, and resistance of the tissue was measured in an Ussing chamber. The authors found that tissue from patients with CRSwNP had profoundly decreased resistance compared with control tissue. This is an important finding because it showed that the *in vivo* barrier function in sinus tissue from patients with CRSwNP was impaired. The Soyka et al study also showed decreased TEER in *ex vivo*-cultured epithelia grown

at ALI from patients with CRSwNP compared with epithelia from control subjects. Although the *in vivo* findings of Soyka et al are in agreement with the present findings, *in vitro* studies using epithelial cells are discordant with our results. As shown in Fig 4, A, we detected no differences in TEER among cells derived from the CRS groups and control subjects, suggesting that barrier dysfunction in patients with CRS is a cell-extrinsic event. Many factors might explain the discrepancy between these findings, including differences in culture technique, nasal scraping technique, anatomic location of the scrapings, timing of the measurements, or levels of OSM *in vivo*, among others.

It is not yet clear which cells are responsible for the increased production of OSM found in diseased mucosal tissues, but ongoing studies in our laboratory are aimed at identifying these cells in patients with CRS. Many types of hematopoietic cells that are known to be present in tissue from patients with CRS have been shown to make OSM *in vitro*, including activated T cells, dendritic cells, mast cells, eosinophils, neutrophils, and macrophages.⁴¹⁻⁴⁴ It would be particularly interesting if M2 macrophages produce OSM in patients with CRS. Our laboratory and others have previously shown that M2 macrophage counts are increased in nasal polyps compared with those in control UT, and these cells produced Factor XIII-A and contributed to excessive fibrin deposition in nasal polyps.⁴⁵⁻⁴⁸ Interestingly, OSM also has been shown to upregulate the production of fibrinogen, which is cleaved into fibrin by thrombin to provide substrate for fibrin deposition.⁴⁹ Thus future studies are needed to determine whether OSM also plays a role in the fibrin deposition and tissue remodeling that occurs in nasal polyps.

Of the mucosal diseases analyzed in this article, the pathogenesis of asthma has been most studied. Asthma is classically thought to involve a predisposition to the development of allergic or type 2 immune responses, leading to persistent inflammation of the airways and subsequent tissue remodeling. However, this allergic/type 2 immunity model does not fully explain many clinical features of asthma, including the heterogeneity of asthma phenotypes and the lack of association between airway eosinophilia and airway hyperresponsiveness.⁵⁰⁻⁵⁴ An alternative model of asthma pathogenesis has been proposed in which the generation of allergy and type 2 immunity is the result of epithelial barrier dysfunction.⁵⁵ This barrier hypothesis is perhaps best supported by the finding that single nucleotide polymorphisms in the skin barrier gene *filaggrin* are associated with development of atopic dermatitis and asthma, suggesting that barrier dysfunction precedes development of atopy or at least sensitization.⁵⁶ Epithelial dysfunction has been characterized in asthmatic patients compared with control subjects. Asthmatic patients have decreased expression of intercellular junction proteins, including occludin, zonula occludens 1, and E-cadherin, and have decreased barrier function assessed based on TEER and dextran permeability.^{7,57,58} Additionally, reduced caveolin-1 expression in airway epithelium from asthmatic patients was shown to associate with decreased E-cadherin expression and promoted epithelial expression of thymic stromal lymphopoietin and IL-33, suggesting that barrier dysfunction might initiate type 2 immune responses.⁵⁷ Thus, therapeutic intervention focused on either preventing barrier dysfunction or restoring barrier function once it is lost might be effective in the treatment of mucosal diseases, including asthma, CRS, and EoE.

Importantly, we have shown that the OSM-mediated decrease in barrier function in cultured NECs from patients with CRS was

reversible (see Fig E2, C), which suggests that therapeutic intervention targeting OSM has the potential to be beneficial in the treatment of CRS through restoration of epithelial barrier function. OSM has been therapeutically targeted in patients with rheumatoid arthritis (RA) through neutralization of OSM and inhibition of the Janus kinase (JAK)/STAT pathway.^{59,60} The JAK inhibitors CP-690,550 and INCB028050, which inhibit JAK-1, JAK-2, and JAK-3, were shown to effectively block the activation of STAT1, STAT3, and STAT5 in OSM-treated fibroblasts *in vitro*.⁶⁰ In contrast, a phase II trial in patients with RA using an anti-OSM mAb, GSK315234, did not show any benefit of blocking OSM, potentially because of low-affinity OSM binding. However, the drug was well tolerated in patients, and the study investigators concluded that a high-affinity anti-OSM antibody could be beneficial in the treatment of RA.⁵⁹ Other potential strategies for the therapeutic neutralization of OSM include using soluble gp130 or receptor fusion proteins. Soluble gp130 is a natural inhibitor of IL-6 family cytokines, and treatment with this protein could potentially be effective in preventing the downstream effects of OSM. However, soluble gp130 is not specific to OSM and would also prevent downstream signaling of other IL-6 cytokine family members, including IL-6, leukemia inhibitory factor, IL-11, and ciliary neurotrophic factor.⁶¹ A receptor fusion protein for the inhibition of OSM has been recently described in a murine model. The receptor fusion protein consists of OSM ligand binding subunits of OSMR and gp130 connected by a flexible linker domain and was shown to be a highly potent and specific inhibitor of OSM.⁶²

In summary, we have shown that OSM levels were increased in the nasal polyps of patients with CRS, profoundly decreased barrier function of airway epithelium *in vitro*, and correlated with epithelial leak in nasal secretions *in vivo*. In addition, our data suggest that OSM levels might be increased in patients with EoE and those with allergic asthma. Thus OSM might play a role in barrier dysfunction in patients with mucosal disease, and inhibition of OSM, its downstream effects, or both might be beneficial in the treatment of diseases associated with type 2 inflammation and loss of barrier function by potentially restoring the barrier function of mucosal epithelial cells.

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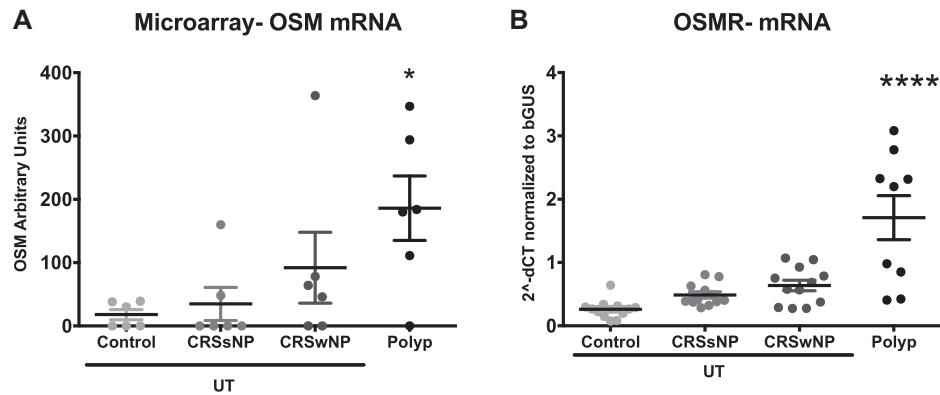


FIG E1. A, OSM expression was increased in nasal polyps compared with that seen in control UT in a microarray analysis (17.83 ± 19.78 vs 186.0 ± 124.8 ; $n = 6$; $P < .05$, ANOVA). **B,** OSMR mRNA expression was increased in nasal polyps and UT from patients with CRSwNP compared with that seen in control UT (1.71 ± 1.04 and 0.64 ± 0.29 vs $0.26 \pm .15$; $n = 8-12$; $P < .0001$, Kruskal-Wallis test). * $P < .05$ and **** $P < .0001$.

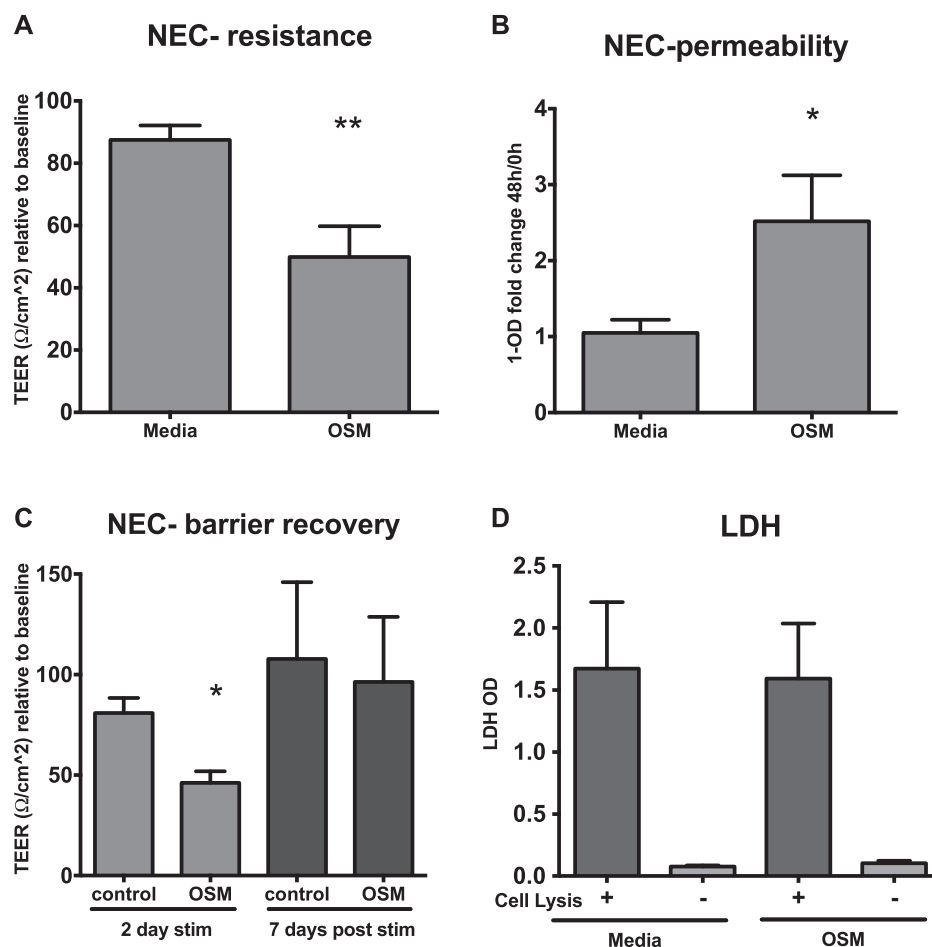


FIG E2. **A**, Fully differentiated NECs from control subjects were left unstimulated or stimulated with 100 ng/mL OSM for 48 hours. OSM stimulation decreased TEER compared with unstimulated control values. Data are presented as the change in resistance over the 48-hour stimulation normalized to resistance at 0 hours to show the percentage of resistance lost during stimulation: $(48\text{-h TEER}/0\text{-h TEER}) \times 100$ ($P < .01$, unpaired t test). **B**, NECs from control subjects were stimulated with 100 ng/mL for 48 hours. OSM-treated cells had an increase in permeability, as measured based on dextran flux ($P < .05$, unpaired t test). **C**, OSM stimulation, 100 ng/mL for 48 hours, decreased barrier function in fully differentiated NECs from the inferior turbinate of patients with CRS, as measured based on TEER. OSM was then removed, and the cells were cultured for 7 more days, at which point barrier function of the previously OSM-treated cells was not different compared with media control values. **D**, OSM stimulation, 100 ng/mL for 48 hours, did not alter cell viability, as measured based on lactate dehydrogenase (LDH) levels compared with lysed cells ($n = 2\text{-}7$). Data are means $\pm$ SEMs. * $P < .05$ and ** $P < .01$.

TABLE E1. Characteristics of subjects providing sinus tissue

	Control subjects			Patients with CRSsNP			Patients with CRSwNP			Patients with CRSwNP, polyps
Total no. of subjects	n = 38 (19 F/19 M)			n = 65 (38 F/17 M)			n = 110 (38 F/72 M)			
Age (y), median (range)	47 (21-75)			39 (19-71)			47.5 (23-72)			
	Y	N	U	Y	N	U	Y	N	U	
Atopy	6	30	2	29	25	11	66	29	15	
Asthma	1	36	1	20	45	0	55	52	3	
Methodology used										
Nasal lavage fluid	n = 10 (6 F/4 M)			n = 12 (7 F/5 M)			n = 41 (10 F/31 F)			
Age (y), median (range)	32 (25-73)			33 (23-56)			53 (22-72)			
Matched nasal secretions/tissue lysate				n = 13 (7 F/6 M)						n = 11 (4 F/7 M)
Age (y), median (range)	47.5 (26-69)			28 (19-69)						42 (33-63)
Tissue lysate	n = 11 (7 F/4 M)			n = 13 (10 F/3 M)			n = 11 (5 F/6 M)			n = 21 (9 F/12 M)
Age (y), median (range)	61 (26-69)			35 (25-71)			53 (24-70)			42 (24-70)
RNA	n = 11 (4 F/7 M)			n = 13 (7 F/6 M)			n = 13 (7 F/6 M)			n = 11 (2 F/9 M)
Age (y), median (range)	45 (20-75)			39 (24-55)			45 (34-65)			48 (23-72)
Cultured NECs	n = 11 (6 F/5 M)			n = 13 (9 F/4 M)			n = 8 (2 F/6 M)			
Age (y), median (range)	45 (20-75)			41 (27-59)			43 (29-64)			
Cultured tissue	n = 4 (2 F/2 M)			n = 10 (5 F/5 M)			n = 9 (5 F/4 M)			n = 23 (7 F/16 M)
Age (y), median (range)	49 (25-72)			40 (23-65)			47 (28-62)			52 (23-72)

F, Female; M, male; N, no; U, unknown; Y, yes.

TABLE E2. Characteristics of subjects providing esophageal tissue

	Total no. of subjects	
	Control subjects n = 8 (7 F/1 M)	Patients with EoE n = 13 (3 F/10 M)
Age (y), median (range)	53.5 (44-59)	36 (27-78)
Eosinophil count, median (range)	0 (0-0)	30 (15-57)