Sinonasal Epithelial Cell Response to *Staphylococcus aureus* Burden in Chronic Rhinosinusitis

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**IMPORTANCE**
Chronic rhinosinusitis (CRS) is an inflammatory disorder of the nose and paranasal sinuses. *Staphylococcus aureus* is increasingly linked with CRS exacerbations. Little is known about how bacteria activate inflammatory pathways that contribute to CRS.

**OBJECTIVE**
To develop an in vitro coculture system to explore how infection with *S aureus* stimulates innate immune responses of sinonasal epithelial cells (SNECs).

**DESIGN, SETTING, AND PARTICIPANTS**
Sinonasal epithelial cells were collected from 13 patients during endoscopic sinus surgery and grown in culture at the air-liquid interface from July 2014 through December 2014.

**INTERVENTIONS**
Differentiated SNECs from control individuals, patients with CRS with nasal polyps (CRSsNP), and patients with CRS without nasal polyps (CRSwNP) were infected with *S aureus* at 3 different concentrations for 24 hours.

**MAIN OUTCOMES AND MEASURES**
Growth of *S aureus* and viability of SNECs were measured. Expression of inflammatory markers and innate immune genes was measured by reverse transcription–polymerase chain reaction. Basal secretion of interleukin 8 was determined by enzyme-linked immunosorbent assay.

**RESULTS**
Cultured SNECs from patients with CRSsNP demonstrated a significant increase (P < .05) in expression of interleukin 8 (23-fold to 82-fold) and tumor necrosis factor (11-fold to 61-fold) at all the tested concentrations of *S aureus*. Control or CRSwNP SNECs demonstrated a significant increase (P < .05) in expression of interleukin 8 (47-fold and 50-fold, respectively) and tumor necrosis factor (106-fold and 58-fold, respectively) at the higher inoculum of *S aureus*. Basal secretion of inflammatory markers correlated with expression changes. No significant changes in expression were observed for the helper T cell, subtype 2, inflammatory mediators tested.

**CONCLUSIONS AND RELEVANCE**
In this study, we developed a model to study early innate immune-mediated changes in SNECs cocultured at an air-liquid interface with bacteria. We also demonstrated that bacterial burden can be detected by SNECs in the absence of adaptive immune-mediated responses. The CRSsNP SNECs are more sensitive to *S aureus* burden than control or CRSwNP SNECs. Future studies will further develop this infection model and explore the SNEC innate immune response to bacteria.

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Chronic rhinosinusitis (CRS) is an inflammatory disorder of the nose and paranasal sinuses that can be broadly divided into 2 groups based on the presence of nasal polyps (CRSwNPs) or the absence of nasal polyps (CRSsNPs). The persistent inflammatory response in CRS involves adaptive and innate components. The adaptive response in both forms of CRS includes a mucosal infiltrate of lymphocytes, neutrophils, and macrophages, but CRSwNPs are differentiated from CRSsNPs by tissue eosinophilia and a helper T cell, subtype 2 (Th2)-biased cytokine milieu, including interleukin 4 (IL-4), IL-5, and IL-13. In addition to these leukocytes, the sinonasal mucosa also harbors resident innate immune cells such as dendritic cells and innate lymphoid cells that contribute to orchestrating the inflammatory response. Finally, sinonasal epithelial cells (SNECs) have a key sentinel role in host defense and in promoting inflammation through production of antimicrobial proteins, cytokines, chemokines, and other inflammatory mediators.

The epithelial cell–derived proteins thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 have been demonstrated to be capable of stimulating Th2 cytokine expression. Interleukin 33 is upregulated in epithelial cell cultures from inflammatory pathway, IL-6, and certain C-X-C cytokines. The role of bacteria in CRS remains unclear. Bacterial infection or colonization that is often seen in CRS may be the primary inflammatory stimulus or could instead occur secondary to a preexisting inflammatory state, perhaps acting only as a disease modifier. Bacterial biofilms, which are sources of persistent bacteria and which serve as a niche for the potential rise of resistant bacteria, have been often found in tissue from individuals with CRS and may serve as reservoirs of infection in treatment-resistant CRS. Pseudomonas aeruginosa and Staphylococcus aureus biofilms are detected more frequently in sinonasal tissue samples from those with CRS than from those without CRS. Colonization with S. aureus, the presence of S. aureus biofilms, and intracellular Staphylococcus have been increasingly linked with CRS exacerbations, suggesting that S. aureus may have a causative role in recalcitrant CRS. The S. aureus biofilms in patients with CRS have been associated with increased expression of inflammasome genes, and in vitro growth of S. aureus on sinonasal tissue explants leads to increased expression of elements of the NOD-2 proinflammatory pathway, IL-6, and certain C-X-C cytokines.

In this study, we developed a coculture system for examining early interactions between SNECs and live bacteria at the air-liquid interface (ALI) and demonstrate that bacterial burden can be detected by sinonasal epithelium in the absence of adaptive immune-mediated responses. This system may provide a basis for further in vitro manipulation to better tease apart the complex interactions between bacteria and the innate immune system and to more fully understand the role of bacterial colonization and infection in CRS.

Methods

Human Participants
The study protocol was approved by the Johns Hopkins Medicine Institutional Review Board, and written informed consent was obtained from all participants. During endoscopic sinus surgery, SNEC tissue was collected from the ethmoid sinuses of 13 patients from July 2014 through December 2014 and grown in culture at the ALI. Three patients were classified as having CRSwNPs, 6 patients were classified as having CRSsNPs, and 4 patients were classified as control individuals. Patients with CRS were defined by having historical, endoscopic, and radiographic criteria and by meeting the definition of the American Academy of Otolaryngology–Head and Neck Surgery Chronic Rhinosinusitis Task Force. Patients had continuous symptoms of rhinosinusitis for more than 12 consecutive weeks that was associated with bilateral mucosal disease on computed tomography of the sinuses. Patients were classified as having CRSwNPs if endoscopy confirmed the presence of bilateral polyps in the middle meatus and were classified as having CRSsNPs if endoscopy excluded polyps in the middle meatus. Controls were defined as those without CRS who were undergoing endoscopic sinonasal surgery for dacryocystorhinostomy, cerebrospinal fluid leak repair, or removal of nonpolyp nasal masses.

Mucosal tissue was transferred to phosphate-buffered saline (PBS), supplemented by penicillin G (100 μg/mL), streptomycin sulfate (100 μg/mL), amphotericin B (2.5 μg/mL), and gentamicin sulfate (50 μg/mL) (all from Gibco). It was processed as described below in the next subsection.

SNEC Culture at the ALI
Sinonasal epithelial cells were grown at the ALI as previously described. Briefly, epithelial cells were isolated from tissue samples by enzymatic degradation and grown in cell culture. Once confluent, cells were trypsinized and then resuspended in bronchial epithelial growth media and plated onto 12-well Falcon filter inserts (0.4-μm pore; Becton Dickinson) coated with human type IV placental collagen (Sigma). When confluent, media was removed from above the cultures, and media below the inserts was changed to LHC basal media: Dulbecco modified Eagle medium–high glucose, pyruvate (50:50; Gibco) containing the same concentrations of additives as bronchial epithelial growth media except that the concentration of epidermal growth factor was reduced to 0.63 ng/mL, and penicillin, gentamicin, streptomycin, and amphotericin B were omitted (ALI media). Each set of SNEC cultures came from a separate patient and was maintained at the ALI, with the apical surfaces remaining free of medium for at least 3 weeks before.
Coculture of SNECs With *S aureus* in Culture

*I Staphylococcus aureus* was obtained commercially (strain 6538; ATCC). The *S aureus* from a frozen stock was grown overnight in Luria broth (Fisher Scientific). An aliquot was resuspended in the SNEC antibiotic-free ALI media described above and then diluted 1:1000 into fresh ALI media or Luria broth and grown at 37°C (300 rpm).

The SNECs were grown in culture in the ALI system described above on antibiotic-free media for at least 3 weeks. The *S aureus* was grown at 37°C (300 rpm) to a density of 10^6 colony forming units (CFUs) per milliliter (log-phase growth) in ALI media and then transferred to the apical surface of the SNECs for 1 hour at various concentrations. The ALI media alone was added to the apical surface of SNECs for 1 hour as a negative control. After 1 hour, the apical supernatant containing bacteria was aspirated, and *S aureus* cocultures with SNECs were grown for 24 hours at 37°C in 5% carbon dioxide.

For wells used for RNA collection or the lactate dehydrogenase (LDH) assay, after 24 hours of growth, the apical chamber was washed in 1× PBS for 10 minutes at 37°C in 5% carbon dioxide. The PBS was collected from the apical surface and centrifuged for 1 minute at 15,000 rpm, and aliquots were used to determine LDH secretion as described in the SNEC death (LDH assay) subsection below. The basal ALI media was collected, spun down for 1 minute at 15,000 rpm, and frozen at −80°C for later use with the enzyme-linked immunosorbent assay (ELISA).

To determine bacterial density, the number of CFUs per milliliter was measured. Then, 300 μL of 1× PBS containing 0.1% Triton X-100 (EMD Bioscience), which will lyse the SNECs but not the *S aureus* (capturing both intracellular and extracellular bacteria), was added to the apical surface, and the SNECs were manually scraped off the inserts into tubes and incubated for 5 minutes at 37°C (300 rpm). Samples were then centrifuged at 10,000 rpm for 1 minute, the supernatant was discarded, and the cell pellet was resuspended in 300 μL of 1× PBS. Aliquots were then used to determine CFUs per milliliter as described below.

Quantifying Bacterial Density

To determine bacterial density, the number of CFUs per milliliter was measured. Then, 100 μL of culture was collected and serially diluted in increments of 1:10 in 1× PBS (Fisher Scientific). Next, 10 μL of each dilution was spotted plated in duplicate or triplicate onto Luria broth–agar (Fisher Scientific) plates, and the plates were incubated at 37°C overnight. Only dilutions that yielded between 10 and 100 colonies were counted, and CFUs per milliliter were calculated as the number of colonies times the dilution factor, divided by the volume plated in milliliters.

SNEC Death (LDH Assay)

The 1× PBS was incubated and then collected from the apical surface of SNECs grown with and without *S aureus* for 24 hours as described above. The LDH release was determined using a commercially available kit (LDH cytotoxicity assay; Pierce) with 50 μL of sample in triplicate in 96-well plates (Costar 9018; Corning) according to the manufacturer’s instructions. Absorbance from each well was read using a microplate reader at 490 nm (signal) and 680 nm (background), and background was subtracted from signal (A490 minus A680). Negative controls (1× PBS and ALI media) and the provided LDH positive control were performed in triplicate for each run.

Real-time Polymerase Chain Reaction

Total RNA was extracted from SNECs following 24 hours of treatment in the presence or absence of *S aureus* using an available kit (RNeasy; Qiagen) according to the manufacturer’s directions. DNase I (Qiagen) was used to treat RNA to remove contaminating genomic DNA. The RNA concentration was determined by measuring the optical density values at 260 nm. Complementary DNA was synthesized from isolated messenger RNA by reverse transcribing 500 ng of RNA in a reaction volume of 20 μL using random hexamer primers (Invitrogen) and reagents from a commercially available kit (Omniscript RT; Qiagen).

Real-time polymerase chain reaction (PCR) analysis was performed using an available system (StepOnePlus; Applied Biosystems) under standard cycling parameters for SYBR green or Taqman per the manufacturer’s recommendations. For IL-8, IL-25, IL-33, and TSLP (eTable in the Supplement), the reaction mix consisted of 50 ng of total RNA (IL-8, IL-25, and TSLP) or 100 ng of total RNA (IL-33), or it consisted of 5 ng of total RNA (18S RNA), 10 μL of SYBR green PCR, 1.5 to 5 mol/L of target primers, or 1.0 mol/L of 18S rRNA primers, in a total volume of 20 μL. Each PCR run was accompanied by housekeeping gene 18S as an internal control and a negative control consisting of all components of the reaction mixture, excluding target RNA. For TNF, the reaction mixture consisted of 100 ng of total RNA, primers (Taqman; Life Technologies) (eTable in the Supplement), and master mix (Taqman Fast Universal PCR; Applied Biosystems) according to the manufacturer’s recommendations. A corresponding 18S Taqman control was also run using 5 ng of total RNA according to the manufacturer’s recommendations. Amplicon expression in each sample was normalized to its 18S RNA content, and the level of expression of target messenger RNA was determined as ACT, the difference in threshold cycles for each target and housekeeping gene. All primers were commercially synthesized (Life Technologies) (eTable in the Supplement).

Enzyme-linked Immunosorbent Assays

Basal secretion of IL-8 was quantified using a commercially available kit (ELISA; eBioscience). Basal ALI media from SNECs from control, CRSsNP, and CRSwNP samples was collected after 24 hours of growth with no *S aureus* or 10^3, 10^5, or 10^7 CFUs/mL. Samples were centrifuged for 1 minute at 15,000 rpm and frozen at −80°C. A human IL-8 instant ELISA kit (BMS204/3INST; eBioscience) was used according to manufacturer’s instructions. Briefly, negative control samples (SNECs grown with no bacteria) were diluted 1:10 in sample buffer, and samples grown with *S aureus* were
Results

Growth of S aureus in SNEC Culture Media
To confirm that S aureus can grow in the SNEC ALI media, S aureus was grown in fresh Luria broth media or fresh ALI media. Colony-forming units per milliliter were determined at various time points. Our results showed that S aureus is able to grow in ALI media (Figure 1A). Growth occurred at a slower rate and to a lower overall density. This is likely related to differences in nutrient levels between the 2 types of media.

S aureus Coculture With SNECs
We next confirmed that S aureus will grow at the ALI of SNECs. The SNECs were grown in culture in the ALI media described above. Staphylococcus aureus was transferred to the apical surface of the SNECs for 1 hour at concentrations of 10^3, 10^5, or 10^7 CFUs/mL. These concentrations of S aureus were chosen because data suggest that there are at least 10^3 CFUs/mL of bacteria present in an acutely infected sinus, as well as to begin to explore if SNECs can detect S aureus in a concentration-dependent fashion.

After 1 hour, CFUs per milliliter were determined for the S aureus remaining on the apical surface, bacteria present in the supernatant, and bacteria grown in ALI media in a culture tube. There was no difference between growth of S aureus in a culture tube and growth of S aureus in the supernatant (Figure 1B). After removal of the supernatant, approximately 10% of the S aureus remained on the apical surface of the SNECs. These data demonstrated that S aureus remains present and can grow on the ALI, which may better mimic growth of bacteria in vivo on sinus mucosa in a nonsubmersed system.

The density of S aureus on the apical surface of the SNECs following the initial 1-hour inoculation with 10^3, 10^5, or 10^7 CFUs/mL was measured following 24 hours of growth (Figure 2A). We observed no difference between growth of S aureus on control SNECs compared with CRS SNECs. These data showed that S aureus can grow equally well on control vs CRS SNECs.

SNEC Cell Death Following Coculture With S aureus
Viability of SNECs was assessed after 24 hours of coculture with S aureus via LDH release from the apical surface as described in the SNEC Death (LDH Assay) subsection of the Methods section. Lactate dehydrogenase is released by dying mammalian cells and is used as a marker of cytotoxicity. There was an increase in LDH secretion as a function of the initial bacterial loading dose compared with growth in the absence of bacteria (Figure 2B). For each initial loading dose of S aureus, no statistically significant difference (P < .05) in LDH secretion was observed between CRS and control SNECs. These data showed that SNEC cell death increases as the bacterial loading dose is increased and further suggested that there is no difference in the overall viability between control and CRS SNECs treated with bacteria.
Absence of Regulation of TH2-Stimulating Cytokines by *S aureus*

We next examined changes in expression of inflammatory mediators in SNECs from control, CRSsNP, and CRSwNP samples following 24 hours of growth with *S aureus* at the ALI. We measured expression of the TH2 mediators of inflammation IL-25, TSLP, and IL-33 (Figure 3), as well as the proinflammatory cytokines TNF and IL-8 (Figure 4). There were no significant changes in expression (P > .05) for TSLP or IL-25 for control, CRSsNP, or CRSwNP SNECs cocultured with bacteria compared with untreated SNECs (Figure 3A and B). No significant difference (P > .05) in expression of IL-33 was observed for control SNECs or CRSwNP SNECs cocultured with bacteria compared with untreated SNECs (Figure 3C). However, CRSsNPs demonstrated a significant increase in IL-33 expression after inoculation with $10^7$ CFUs/mL of *S aureus* (P = .05).
Expression of Proinflammatory Cytokines With a Smaller Burden of S aureus by CRSsNP SNECs

Expression of the proinflammatory cytokines TNF and IL-8 was also examined to assess the ability of this system to detect changes in epithelial cell activity as a function of coinfection. Tumor necrosis factor and IL-8 are important mediators of inflammation and have a role in early recruitment of neutrophils and other lymphocytes in response to bacterial infection of mucosal surfaces.12,13 Upregulation of these cytokines has been demonstrated in a submersed tissue culture model of CRS epithelial cells treated with Streptococcus pneumoniae,23 and varying levels of these cytokines have been demonstrated in tissue and mucus from individuals with CRS.10 Baseline expression of TNF and IL-8 was similar among SNECs from control, CRSsNP, or CRSwNP samples (Figure 4A and C). For control SNECs, there was a trend toward increased expression of TNF as a function of initial bacterial burden (range, 6-fold to 106-fold increase); however, a significant increase in expression (P < .05) was only seen following coculture with 107 CFUs/mL of S aureus. A similar trend of increased TNF expression following coculture with S aureus was also observed with CRSwNP SNECs (Figure 4C).

The CRSsNPs displayed a significant increase in expression of TNF after coculture with all initial loading densities of S aureus (Figure 4C). Furthermore, there was a significant increase (P = .02) in TNF expression between CRSsNP and control SNECs following coculture with 103 CFUs/mL (5-fold difference in expression) or 105 CFUs/mL (8-fold difference in expression) of S aureus.

A similar pattern of expression changes was observed for IL-8 expression with respect to control, CRSsNP, and CRSwNP SNECs (Figure 4B and D). For control SNECs, there was a significant increase in expression (P < .05) of IL-8 (range, 3-fold to 46-fold increase) as a function of initial bacterial burden following coculture with 103 CFUs/mL or 105 CFUs/mL of S aureus (Figure 4B). Again, CRSsNPs displayed a significant increase in expression of IL-8 after coculture with all initial loading densities of S aureus (Figure 4D). Furthermore, there was a significant increase (P = .02) in IL-8 expression between CRSsNP and control SNECs following coculture with 103 CFUs/mL (11-fold difference in expression) and a near-significant increase in expression (P = .06) with 105 CFUs/mL (6.8-fold difference in expression) of S aureus. No difference (P > .05) was observed in expression of IL-8 between CRSwNP and control SNECs cocultured with S aureus.
These data demonstrated that, as has been shown in other mucosal systems, our system is capable of detecting changes in inflammation associated with bacterial infection. Furthermore, our data suggested that CRSsNP SNECs are more sensitive to S. aureus burden and further indicated that CRSwNP cells behave more like controls, which is consistent with more of a Th2-type response driving CRSwNPs compared with CRSsNPs.

**Secretion of Proinflammatory Cytokines With a Smaller Burden of S. aureus by CRSsNP SNECs**

To confirm that changes in expression of proinflammatory cytokines result in changes in secretion of cytokines, we measured secretion of IL-8 into the basal compartment (see the Enzyme-linked Immunosorbent Assay subsection of the Methods section). Secretion into the basal compartment was chosen because movement of cytokines in this direction in vivo would propagate signals into the underlying stromal tissue and result in recruitment of neutrophils, macrophages, and other lymphocytes. We found that secretion of IL-8 into the basal compartment is significantly increased \( (P < .05) \) for CRSsNP SNECs, from a basal secretion of 4 ng/mL to 16 ng/mL with \( 10^3 \) CFUs/mL of S. aureus, to 30 ng/mL with \( 10^5 \) CFUs/mL of S. aureus, and up to 60 ng/mL with \( 10^7 \) CFUs/mL of S. aureus \( (\text{Figure 5}) \). Control and CRSwNP SNECs exhibited a significant increase \( (P < .05) \) in IL-8 secretion only after exposure to \( 10^7 \) CFUs/mL of S. aureus. Control SNECs secreted IL-8 (62 ng/mL) at a level comparable to that of CRSsNPs (60 ng/mL) at this high bacterial burden compared with CRSwNPs, which secreted less IL-8 (31 ng/mL). These data supported the premise that CRSsNP SNECs are more sensitive in their response to S. aureus than control or CRSwNP SNECs.

### Discussion

In this study, we developed an SNEC coculture system to study early innate immune-mediated responses of SNECs that are associated with bacterial infection. We demonstrated that SNECs can detect S. aureus burden and that SNECs derived from patients with CRSwNPs are more sensitive to S. aureus burden than control or CRSwNP SNECs. The epithelial cell response elicited by S. aureus appears to be primarily directed toward stimulating recruitment of neutrophils and lymphocytes through production of IL-8 and TNF. This is consistent with the histology of CRS, which is characterized in part by neutrophil and lymphocyte infiltrates and fibrosis, with CRSwNPs also having an eosinophilic component.

In the coculture model system, the apical surface of the differentiated epithelial cells is submerged in media containing S. aureus to allow time for bacteria to settle. The apical liquid is then removed, and the remaining bacteria are left exposed at the ALI. Biofilm formation with P. aeruginosa has been demonstrated at the ALI in a mouse SNEC model, and it is possible that S. aureus forms biofilms in our system under the harsher conditions of the ALI. We also observed that the final S. aureus densities are similar after 24 hours of growth, implying that the inoculating density and time of exposure to higher densities of bacteria are driving the observed inflammatory changes (Figures 3, 4, and 5). Despite the fact that SNECs grown in culture lack external signals from the host adaptive immune system, our results demonstrated that stimulation of SNECs by S. aureus can be stratified by the clinical phenotypes of control, CRSsNP, and CRSwNP samples. This suggests that SNECs have inflammatory memory and that CRSsNP epithelial cells are primed to respond to lower levels of bacteria. While the individual innate immune components (eg, Toll-like receptors and other pattern recognition receptors) lack antigen specificity or immunologic memory, it is possible that the SNECs maintain an altered but stable pattern of expression and production of innate immune mediators, perhaps as a result of chronic maladaptive inflammatory changes.

The CRSwNP SNECs exposed to a high burden of S. aureus secreted significantly less IL-8 compared with control or CRSsNP SNECs \( (\text{Figure 5}) \). Our results suggested that, in contrast to CRSsNP epithelial cells, CRSwNP epithelial cells are unable to mount a robust inflammatory response to bacteria, which agrees with previous research that suggests innate antimicrobial responses are blunted in CRSwNPs, perhaps predisposing to bacterial or fungal overgrowth.

Treatment of CRS exacerbations often begins with a trial of antibiotic therapy, which in theory is aimed at reducing the overall bacterial burden. In this study, CRSsNP SNECs activated inflammatory pathways at a lower bacterial burden than control or CRSwNP SNECs. In an acute setting, the ability to detect bacteria at a lower threshold may be beneficial by generating an appropriate cytokine response and
subsequent host inflammatory cascade that could theoretically reduce the need for extrinsic antimicrobial agents to help eradicate an infection. However, in chronic inflammatory conditions, repetitive stimulation with an irritant can lead to pathologic changes in the affected tissue, as well as maladaptive inflammatory responses that sustain a chronic inflammatory state. It is possible that, as a result of chronic inflammation in patients with CRSsNPs, these epithelial cells may maintain the ability to detect and trigger inflammatory responses with lower bacterial burdens, which results in a vicious cycle of sustained inflammation. This implies that a greater reduction of bacterial burden may be needed to alleviate bacteria-driven inflammation in patients with CRSsNPs. Furthermore, eradication of pathogenic bacteria may be more difficult in patients with CRS, in whom bacteria can exist in more exclusive niches (biofilms or in intracellular compartments). As such, current paradigms of antibiotic use that are effective in most cases of acute bacterial sinusitis in controls may not effectively reduce the burden of bacteria to subinflammatory levels in bacterial CRS exacerbations.

Irrespective of the role of bacteria as a causal factor for CRS, antibiotics continue to have a central role in treatment of active CRS. While data suggest that certain classes of antibiotics have direct anti-inflammatory properties, the end point of antibiotic treatment is to reduce the burden of bacteria. In this study, we demonstrated that bacterial burden can be detected by SNECs in the absence of adaptive immune-mediated responses. Furthermore, CRSsNP SNECs activated an inflammatory response at a lower initial inoculum of S aureus than control or CRSwNP SNECs. This implies that a greater reduction of bacterial burden with antimicrobials may be needed to alleviate bacterially mediated inflammation in patients with CRSsNPs.

Conclusions

In an ALI coculture model system, bacterial burden can be detected by differentiated SNECs in the absence of adaptive immune-mediated responses. Furthermore, CRSsNP SNECs are more sensitive to S aureus density than control or CRSwNP SNECs. The increased sensitivity of CRSsNP SNECs may be the result of stable, maladaptive changes to the innate inflammatory response pathways that help maintain a vicious cycle of sustained inflammation secondary to exposure to a bacterial irritant. Future studies will explore these ideas, as well as the SNEC innate immune response to bacteria, and further develop this coculture infection model.

REFERENCES


