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Th2 cytokines associated with chronic rhinosinusitis with polyps down-regulate the antimicrobial immune function of human sinonasal epithelial cells

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Abstract

Background—Chronic rhinosinusitis with nasal polyps (CRSwNPs) is a disorder characterized by persistent eosinophilic Th2 inflammation and frequent sinonasal microbial colonization. It has been postulated that an abnormal mucosal immune response underlies disease pathogenesis. The relationship between Th2 inflammatory cytokines and the innate immune function of sinonasal epithelial cells (SNECs) has not been explored.

Methods—Human SNECs (HSNECs) isolated from control subjects and patients with CRS were assessed for expression of antimicrobial innate immune genes and proinflammatory cytokine genes by real-time polymerase chain reaction, ELISA, and flow cytometry. A model of the Th2 inflammatory environment was created by exposure of primary HSNEC to the Th2 cytokine interleukin (IL)-4 or IL-13 for 36 hours, with subsequent assessment of innate immune gene expression.

Results—HSNEC obtained from CRSwNP patients displayed decreased expression of multiple antimicrobial innate immune markers, including toll-like receptor 9, human beta-defensin 2, and surfactant protein A. Baseline expression of these genes by normal and CRS HSNEC in culture is significantly down-regulated after incubation with IL-4 or IL-13.

Conclusion—Expression of multiple innate immune genes by HSNEC is reduced in CRSwNP. One mechanism appears to be a direct effect of the leukocyte-derived Th2 cytokines present in the sinonasal mucosa in CRSwNP. Impaired mucosal innate immunity may contribute to microbial colonization and abnormal immune responses associated with CRSwNP.

Keywords

Chronic; epithelial; innate; innate immunity; nasal polyps; polyps; rhinosinusitis; Th2 cytokines; TLR9

Although significant progress has been made in the treatment of sinonasal inflammatory disease, chronic rhino-sinusitis with nasal polyps (CRSwNP) remains an entity that is

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particularly difficult to treat.^{1–3} The clinical behavior of CRSwNP often defies the classic concepts underlying current management strategies in rhinosinusitis. Specifically, surgical reestablishment of sinus ostial patency and clearing of infection within the sinus cavities does not necessarily reverse the disease process and restore normal function in CRSwNP. Although, in general, the majority of sinusitis cases do respond to this proven therapeutic approach, those that fail tend to be characterized by persistence of mucosal inflammation, despite widely patent sinus openings, and a continued microbial presence, despite adequate antimicrobial coverage. For these recalcitrant forms of sinusitis, research is clearly needed to understand the basic pathophysiology and to develop novel treatment alternatives.

In many respects, recalcitrant CRSwNPs appears to be an inflammatory disorder of the local mucosal immune system. The sinonasal mucosa is a first site of contact between the host and the outside environment and consequently has a critical role in host defense.⁴ Many microorganisms enter the sinonasal tract during the act of breathing, but multiple innate immune mechanisms normally serve to remove potential pathogens.^{5–7} Through secretion of antimicrobial products, mucociliary clearance, and activation of appropriate adaptive immune processes, the sinonasal epithelial lining is able to create an environment that is inhospitable to infecting organisms. However, in CRSwNP, these protective functions seem to be diminished, permitting microbial colonization within the nose and sinus cavities. This perceived persistence of infection in CRSwNPs is frequently the impetus for repeated medical and surgical therapy. Although several factors might contribute to impaired mucosal immunity, our previous research suggests that there is a relative decrease in expression of innate immune genes related to microbial recognition by sinonasal epithelial cells. Failure of epithelial cells to identify potential pathogens at the mucosal surface and respond vigorously and immediately to them may contribute to the development of microbial colonization in CRSwNP. In turn, epithelial “barrier defects” have been postulated to activate proinflammatory adaptive immune mechanisms in other mucosal-based human diseases.^{8,9}

In previous studies, we have shown that messenger RNA for pattern recognition receptors known as toll-like receptors (TLRs) is expressed in sinonasal mucosa, both in health and in sinus disease.^{10,11} Activation of TLRs on sinonasal epithelial cells (SNECs) induces expression of innate immune effector genes and signaling molecules such as defensins, complement components, and interleukin-8 (IL-8).¹² TLRs are transmembrane receptors that interact with pathogen ligands through an extracellular domain and signal the presence of the microbial products through an intracellular domain.¹³ At least 10 TLRs that recognize specific pathogen molecules are expressed in the sinonasal mucosa, each of which is thought to play a role in the innate immune response to both innocuous microbes in the sinonasal cavity as well as airborne bacterial, fungal, or viral pathogens.¹⁴ Among the TLRs, we have been particularly interested in TLR9, in which its ligand is bacterial or viral unmethylated CpG DNA. TLR9 is strongly expressed in normal nasal epithelium, and its expression is significantly down-regulated in the SNEC of patients with CRSwNP.^{15,16} In addition, activation of TLR9 by CpG is known to be Th1 polarizing and capable of suppressing Th2 inflammation. Given that CRSwNP is a Th2-biased eosinophilic inflammatory condition characterized by bacterial colonization, these features of TLR9 biology suggest a possible role in CRSwNP pathogenesis. We have hypothesized that underactivity of the innate immune system in the sinonasal mucosa may play a role in the perpetuation of Th2 inflammation and the failure to restore Th1–Th2 balance in CRSwNP. Moreover, relative down-regulation of TLR9 in recalcitrant CRSwNP may contribute to the colonization of the sinonasal cavities with bacteria and fungi.

A distinguishing feature of CRSwNP is the predominance of eosinophils and a Th2 cytokine profile in the sinus mucosa.³ The basis for this immune phenotype is unknown, and both host and exogenous mechanisms have been proposed. The effect of an established Th2-biased inflammatory mediator environment on the function of the human sinonasal epithelium has

not been investigated directly. There is increasing evidence that a Th2 cytokine milieu may inhibit innate antibacterial activity at mucosal surfaces. For example, in human intestinal epithelial cells, Mueller *et al.* indicated that Th2 cytokines suppress antimicrobial immunity.¹⁷ Similarly, Beisswenger *et al.* showed that pulmonary antibacterial host defense was inhibited in a murine model of allergic airway inflammation.¹⁸ Further evidence of a down-regulation of innate immunity in Th2 nasal inflammation is provided by a study by Kirtsreesakul *et al.* examining clearing of *Streptococcus pneumoniae* infection in allergic mice.¹⁹ It was found that nasal allergen-sensitized BALB/c mice (in which a Th2 inflammatory response is favored) were less able to clear infection than C57BL/6 mice (which favor a Th1 response). Our previous observation that TLR and immune effector genes are down-regulated in recalcitrant CRSwNP patients is consistent with a similar suppression of epithelial innate immune function by Th2 cytokines within the sinonasal mucosa. At the same time as antibacterial innate immune genes are down-regulated in recalcitrant CRSwNP, we also have found that “antiparasite” or proeosinophilic genes, such as acidic mammalian chitinase, are up-regulated.²⁰ We propose that Th2 cytokines present in CRSwNPs direct the innate immune activity of epithelial cells into an antiparasite program of gene expression at the expense of the Th1-induced antimicrobial pattern. In this way, Th2 inflammation within the sinonasal epithelium may create a permissive environment for microbial colonization.

METHODS

Human Subjects

Thirty-two patients with CRS and 10 control subjects were enrolled in the study. The research protocol was approved through the Johns Hopkins Institutional Review process, and all subjects gave signed informed consent. The CRS subjects were classified into CRSwNP and chronic rhinosinusitis without nasal polyps (CRSsNP) groups as defined by historical, endoscopic, and radiographic criteria and by meeting the definition of the American Academy of Otolaryngology–Head and Neck Surgery Chronic Rhinosinusitis Task Force.³ Specifically, the patients had continuous symptoms of rhino-sinusitis as defined by the Task Force report for >12 consecutive weeks, associated with computed tomography of the sinuses revealing isolated or diffuse sinus mucosal thickening or air–fluid levels. CRSwNP patients had at least one sinonasal polyp present on nasal endoscopy before or at the time of endoscopic sinus surgery. Surgery was performed only if a patient’s symptoms and radiographic findings failed to resolve, despite at least 6 weeks of treatment with oral antibiotics, topical corticosteroids, decongestants, and mucolytic agents, as well as 4 weeks of systemic corticosteroid.

After surgery, CRSwNP patients underwent endoscopic surveillance at regular intervals as per the normal practice of the senior investigator. Patients with recurrence or persistence of polyps after at least 6 months after the surgery, despite a standard postoperative medical regimen including topical corticosteroids, saline lavage, and allergy therapy as indicated, were further subclassified as having “recalcitrant” disease.

The control subjects had no evidence of sinus disease and were undergoing endoscopic approaches for orbital decompression, cerebrospinal fluid leak repair, or sphenoidotomy for biopsy of an isolated, noninflammatory sphenoid sinus process. Before surgery, both the CRS patients and the control subjects received 1 week of oral methylprednisolone and oral antibiotics. All tissue specimens were taken from the resected uncinate process and anterior ethmoid sinus. The specimens were immediately placed in saline on ice and transported to the laboratory for epithelial cell isolation and culture.

Epithelial Cell Dissociation from Human Sinus Mucosal Tissue

Mucosal tissue removed during endoscopic sinus surgery was collected into sterile cold saline and transferred to phosphate-buffered saline (PBS) supplemented by penicillin (100 U/mL; Gibco, Gaithersburg, MD), streptomycin (100 μ g/mL; Gibco), amphotericin B (2.5 μ g/mL; Gibco), and gentamicin (50 μ g/mL; Gibco). The samples were placed through a cell strainer (BD Falcon, Sigma, St. Louis, MO) into Ham's F12 media containing 0.01% protease Sigma type XIV (Sigma) and supplemented with antibiotics as above. Digestion in protease was performed at 4°C overnight, with separation of cells by agitation the following day. The cells were separated by straining into a conical tube to which fetal bovine serum (FBS) was added to a final concentration of 10%, inactivating the protease. The cells were centrifuged twice at 1300 rpm for 10 minutes, after which the supernatant was aspirated and discarded. The washed passage 0 (P0) epithelial cells were then seeded, at a density of $\geq 1.5 \times 10^4$ cells/cm², onto Vitrogen 100-coated (1:75 in sterile water; Cohesion, Palo Alto, CA) P-100 dishes in bronchial epithelium growth medium (BEGM) as previously described.²¹ The cells were then grown at 37°C for 24 hours and then washed with Hanks Balanced Salt Solution (HBSS; Biofluids, Rockville, MD) to remove debris. The media was then changed every 48 hours until the cells reached confluence.

SNEC Culture at the Air–Liquid Interface (ALI)

Confluent cells were washed with HBSS before trypsinization and then treated at 37°C for 4 minutes with a solution containing 0.2% Trypsin (Sigma), 1% polyvinylpyrrolidone (Sigma), and 0.02% EGTA (Sigma) in HBSS. The trypsin was then neutralized by the addition of an equal volume of cold soybean trypsin inhibitor at a concentration of 1 mg/mL in Ham's F12 media. Dissociated cells were washed and resuspended into BEGM media and plated into human type IV placental collagen (type VI; Sigma) coated 6-well Falcon filter inserts (0.4 μ m pore size; Becton Dickinson, Franklin Lakes, NJ). The P1 cells were grown to confluence with BEGM, above (1 mL) and below (2 mL) the cells. When confluent, medium is removed from above the cultures and the medium below the inserts was changed to ALI medium consisting of LHC Basal Medium/DMEM-H (50:50; Gibco) containing the same concentrations of additives as BEGM with the exception that the concentration of epidermal growth factor is reduced to 0.63 ng/mL, and amphotericin B is omitted. Each set of cultures came from a separate patient source and was maintained at the ALI with the apical surfaces remaining free of medium for at least 3 weeks before study.

Stimulation of Cells

Ciliated epithelial cells at the ALI were stimulated apically with 5–25 ng/mL of IL-4 or IL-13 or with 50 ng/mL of IFN- γ diluted in ALI medium for 36 hours. In other experiments, cells were exposed to 10 μ mol of TLR9 agonist (invivogen). Control wells were stimulated with 0.5 mL of ALI medium alone. In the flow cytometry experiment, cells were treated with 10 ng/mL of IL-4 and IL-13.

Flow Cytometric Analysis of Intracellular TLR9 Expression

Adherent cells at the ALI were detached from 6-well inserts as described previously and transferred into 1.5-mL microfuge tubes at a concentration of 1×10^6 cells/tube. Cells were first fixed and permeabilized using a kit from eBiosciences (San Diego, CA). Briefly, cells were fixed using 100 μ L of fixation buffer for 20 minutes at room temperature followed by two washes in permeabilization buffer. Permeabilized cells were then incubated with either 0.5 μ g of phycoerythrin-conjugated TLR9 or rat anti-human IgG isotype control (eBiosciences) for 30 minutes at 4°C. Cells were then centrifuged and washed twice with cold PBS with sodium azide and 2% FCS and resuspended in PBS. All cells were also stained with FITC anti-epithelial cell antigen (Dako, Carpinteria, CA) to verify purity of epithelial cells. Analysis was performed

on a FACScalibur flow cytometer and data were analyzed using CellQuest software (BD Biosciences, San Jose, CA). Cell surface protein is expressed as the increase in mean fluorescence intensity over background (species-specific isotype-matched control) or percentage of positive staining cells.

RNA Extraction/Reverse Transcription

Total RNA was isolated with RNeasy Mini kit (Qiagen, Valencia, CA) using the manufacturer's protocol. RNA was quantified spectrophotometrically and absorbance ratios at 260/280 nm were >1.80 for all samples studied. Five hundred nanograms of total RNA was reverse transcribed in a 20- μ L volume with random hexamer primers (Invitrogen, Carlsbad, CA), 20 U of RNase inhibitor (Applied Biosystems, Foster City, CA), and Omniscript RT kit (Qiagen) under conditions provided by the manufacturer.

Real-Time Polymerase Chain Reaction (PCR)

Real-time PCR was performed in a Light-Cycler 1.2 (Roche Applied Science, Indianapolis, IN) using the SYBR Green PCR Kit (Qiagen). The 18S (sense, 5'-GTAACCCGTTGAAC-CCCAT-3'; antisense, 5'-CCATCCAATCGGTAGTAGCG-3') was used as a internal control. The reaction mix consisted of 50 ng of cDNA (target genes) or 5 ng of cDNA (18S RNA), 10 μ L of QuantiTect SYBR Green PCR, and 0.5 mol/L of primers in a total volume of 20 μ L. All primers were commercially synthesized by Invitrogen. The cycle parameters used were 95°C for 15 minutes to activate Taq polymerase, followed by 35 cycles at 94°C for 15 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. Amplicon expression in each sample was normalized to its 18S RNA content. The level of expression of target mRNA was determined as Δ CT. The Δ CT method uses the difference in CT value obtained between normalizing housekeeping gene (18S) and target gene to calculate relative quantification (Δ CT = the difference in threshold cycles for target and housekeeping gene). This normalization reduces sample-to-sample variations in signal strength. A decrease in the Δ CT by 1 U equals a doubling of the level of the target gene. Consistent use of cDNA described previously (50 and 5 ng for target molecules and 18S RNA, respectively) resulted in highly reproducible real-time PCR cycle thresholds for each of the amplicons across all cell samples. Negative controls, consisting of reaction mixtures containing all components except target cDNA, were included in each PCR run.

ELISA of IL-6, IL-8, Eotaxin-3, and Human β -Defensin 2 (HBD-2)

HBD-2, eotaxin-3, IL-6, and IL-8 released to culture medium were quantified using ELISA according to the manufacturer's instructions (IL-6, IL-8, and eotaxin-3 from BD Biosciences; HBD-2 from Peprotech, Rocky Hill, NJ).

Statistical Analysis

Raw data from real-time PCR were entered into a spreadsheet (Excel; Microsoft Corp., Redmond, WA). Statistical analysis was performed using software (Excel). Data are expressed as mean $\pm$ SEM. Statistical significance of differences was determined using either a two sample *t*-test assuming unequal variances for parametric data, or a Wilcoxon signed rank test for nonparametric paired data. Differences were considered statistically significant at $p < 0.05$.

RESULTS

SNECs were obtained from 9 control subjects and 19 CRSwNP patients in the initial set of experiments. mRNA was extracted after growth in culture at the ALI. The CRSwNP patients were subdivided into recalcitrant and responsive groups (10 subjects and 9 subjects, respectively) based on endoscopic evidence of recurrence or persistence of polyps at least 6

months after surgery. Real-time PCR analysis revealed decreased expression of mRNA for the innate immune proteins HBD-2, mannose binding lectin, and surfactant protein A in the recalcitrant CRS groups versus controls (Fig. 1). In addition, flow cytometry showed decreased basal expression of TLR9 in cultured HSNEC from CRSwNP patients when compared with controls (Fig. 2).

To investigate the impact of the cytokine milieu in CRSwNP on the expression of TLR9 by HSNEC, we used an epithelial cell culture model system. We obtained HSNEC from control subjects and from grossly uninflamed regions of sinus mucosa in CRSsNP patients. We first examined the effect of IFN- γ , a prototypical Th1 cytokine on TLR9 protein expression. When cultured HSNECs from five control subjects were stimulated with IFN- γ , there was an increase in TLR9 by an average of 49.8%, as detected by flow cytometry, compared with unexposed cells from the same patient (Fig. 3). We assessed the functionality of the TLR9 receptor on HSNECs from five control subjects by exposure to its agonist, CpG DNA (10 μ mol) for 36 hours. Evaluation of supernatants for HBD-2 and IL-8 protein revealed induction of both genes after TLR9 stimulation (Fig. 4). The induction of HBD-2 achieved statistical significance ($p = 0.03$), whereas IL-8 did not ($p = 0.1$).

In additional experiments designed to simulate the cytokine milieu in CRSwNP, cultured HSNECs from four control and four CRSsNP subjects were exposed to a combination of IL-4 and IL-13. These Th2 cytokines had the opposite effect to interferon- γ , with an average decrease in TLR9 expression by 46.6% (Fig. 5). Supernatants from these cultures were collected and analyzed by ELISA along with an additional four control and nine CRSsNP patient cultures. The expression of secreted IL-6, IL-8, HBD-2, and eotaxin 3 proteins was assessed by ELISA in HSNECs exposed to IL-4 and IL-13 alone or in combination. Pilot experiments revealed that both cytokines had approximately the same effect (data not shown); therefore, the remaining experiments used IL-4 only. In both control and CRSsNP patients ($n = 10$), it was found that IL-4 reduced expression of IL-6, IL-8, and HBD-2, while very significantly inducing expression of eotaxin 3 (Fig. 6). The magnitude of the effect did not vary between HSNECs derived from control and CRS subjects (data not shown). In 10 HSNEC cultures from individual subjects, real-time PCR was used to determine the effect of IL-4 on expression of surfactant protein A and TLR9 mRNA. This analysis revealed a significant decrease in the expression of TLR9 mRNA ($p = 0.01$) and a trend toward decreased SPA mRNA after IL-4 treatment ($p = 0.2$; Fig. 7).

DISCUSSION

In this study, we presented a novel discovery that Th2 cytokines, in particular IL-4, can modulate the expression of innate immune genes by SNECs. Our findings suggest that IL-4 acts on SNECs *in vitro* to down-regulate production of TLR9, SPA, HBD-2, IL-6, and IL-8. Epithelial cells derived from patients with recalcitrant CRSwNP displayed decreased expression of SPA, HBD-2, and mannose binding lectin. In contrast, the Th1 cytokine IFN- γ increases TLR9 expression, and CpG DNA (TLR9 ligand) stimulates production of multiple innate immune effectors and pro-Th1 chemokines. Thus, the antimicrobial immune activity of HSNECs may be directed, at least in part, by the local cytokine milieu. Because CRSwNP is characterized by Th2-biased inflammation, the resulting down-regulation of antimicrobial mucosal immunity could then contribute to the chronic state of sinonasal infection or microbial colonization. Although Th2 cytokines blunt the antimicrobial response of SNECs, they also induce expression of proeosinophilic mediators, such as eotaxin and acidic mammalian chitinase.²⁰ Taken together, these relationships suggest that an interaction exists between the adaptive immune system and the innate immune function of the epithelial cells that line the sinonasal mucosa. The local Th1/Th2 cytokine environment influences the nature of the innate

immune response, perhaps helping to explain the permissive environment for microbial colonization found in CRSwNP.

The initiating factors that underlie persistent inflammation and microbial colonization in CRSwNP are not well understood. Although Th2 inflammation is a central characteristic of the active disease process, what triggers the local production of Th2 cytokines and infiltration of lymphocytes and eosinophils in the first place is unknown. Multiple groups have proposed that CRS results from an abnormal immune response to microorganisms or their products, including fungi, *Staphylococci*, *Pseudomonas* biofilms, or viruses.^{22–29} Innocuous or ubiquitous agents do not ordinarily generate a vigorous immune response in healthy hosts, and normal mucosal immune activity against potential microbial pathogens typically involves elaboration of innate immune effectors and Th1-like inflammatory mediators. In CRSwNP, the mucosal immune abnormality appears to be twofold: on the one hand, there is a failure to prevent or eliminate microbial colonization of the sinonasal cavities, and on the other hand, there is a seemingly inappropriate Th2-biased immune response perpetuated within the mucosa. The results of this study would suggest that these issues are related, *i.e.*, that the latter contributes to the former. Whether or not the opposite is true—that exogenous agents or microorganisms can drive the mucosal immune response in a Th2 direction—is an area of current investigation.

As an initial point of contact between the host and the outside world, the sinonasal tract is constantly exposed to microorganisms. There are constitutively active mechanisms that serve to keep the growth of “normal flora” in check and clear microbes to the nasopharynx. The transitory presence of organisms generally is tolerated by the mucosal immune system, and this does not elicit a significant inflammatory reaction. In an analogous manner, multiple bacterial species colonize the mucosa of the intestines, where they coexist commensally with the host. In both the intestine and the airway, experimental evidence suggests that Th2 cytokines inhibit innate immune activity of the epithelium and may promote bacterial overgrowth. *In vitro* experiments with human intestinal epithelial cells reveal that Th2 cytokines down-regulate TLR expression and inhibit TLR signaling function.¹⁷ In human bronchial epithelial cells, exposure to Th2 cytokines also blunts the innate immune response to *Pseudomonas*.¹⁸ Although no mouse model currently exists for CRSwNP, mice with allergic Th2 inflammation of the upper and lower airways have been generated. In both cases, airway Th2 inflammation leads to decreased expression of innate immune genes and reduced capacity to clear experimentally induced infection.^{18,19} Taken together, these studies strongly support the validity of the *in vitro* findings with HSNECs that we have described.

Multiple lines of evidence suggest that dysregulation of the complex interaction between the host and microbial agents at the sinonasal mucosal surface may underlie CRSwNP. A cause-and-effect relationship between Th2 inflammation and microbial colonization might explain the presence of chronic infection and biofilms within the nose and sinuses in polypoid rhinosinusitis. The opposite causal relationship is conceivable also, although to date there is little evidence that infectious agents can directly elicit Th2-type inflammation of the type seen in CRSwNP. Nonetheless, because epithelial cell innate immune function may be modulated by cytokines, it is possible that dysregulation of this same pathway may result in diminished antimicrobial function even in the absence of Th2 cytokines. We speculate that exogenous stimuli exist that bind to yet unidentified pattern recognition receptors on epithelial cells, leading to such a proeosinophilic, or “antiparasite,” program of gene expression.^{4,20} Dysregulated activity of an epithelial cell-driven, Th2-biased, mucosal innate immune response would be consistent with the characteristic clinico-pathological features of CRSwNP (Fig. 8).

Maintenance of mucosal homeostasis is a host defense function involving cooperation between multiple integrated immune components. Clearly, studying SNECs in culture imperfectly models the immune function of these cells *in vivo*. It is important to recognize that the cells lack their normal interactions with other resident cell populations and mediators, which may modulate their function and gene activity. Also, growth in culture, in and of itself, may lead to phenotypic changes that affect innate immune gene expression. We are actively exploring these issues in ongoing studies with acutely isolated brushings of epithelial cells and by developing *in vivo* animal models of Th2 sinonasal inflammation. Additional research will be needed to better define the precise role by which cytokines modulate TLR9 and other innate immune effector levels at the transcriptional level. A better understanding of the sinonasal mucosal innate immune system and its interaction with microbes and their products has the potential to result in new approaches to the management of recalcitrant CRSwNP.

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Figure 1.

Cultured SNECs from patients with recalcitrant CRSwNP have diminished baseline mRNA expression of HBD-2, surfactant protein A, and mannose binding lectin. SNECs isolated from patients with and without CRSwNP were grown in cell culture for 2–3 weeks before harvesting of cells for flow cytometry and extraction of mRNA. The level of expression of mRNA for mannose-binding lectin, HBD-2, and SPA was measured by real-time PCR. In each case, expression was reduced in recalcitrant CRS versus controls. (A) SPA, *0.08; (B) HBD-2, *0.04; (C) MBL, *0.04. Columns represent means $\pm$ standard error of the mean.

Figure 2.

Cultured SNECs from patients with recalcitrant CRSwNP have diminished baseline expression of TLR9 protein. In fixed and permeabilized SNECs from control subjects, intracellular TLR9 was evident in 49–91% of cells. In contrast, TLR9 expression was shown in 11–16% of cultured sinonasal cells from 10 CRSwNP patients. This difference was statistically significant ($p = 0.005$).

Figure 3.

IFN- γ stimulation increases TLR9 expression in cultured HSNECs. The Th-1 promoting cytokine, IFN- γ , increases TLR9 expression by SNECs derived from control subjects by an average of 49.9%. This figure depicts an example of a flow cytometry histogram plot from one subject.

Figure 4.

Expression of HBD-2 and IL-8 by cultured HSNECs is increased by exposure to TLR9 agonist. Exposure to TLR9 agonist in vitro induces expression of HBD-2 and IL-8 protein as assessed by ELISA (* $p = 0.03$, Wilcoxon signed rank; columns represent means $\pm$ standard error of the mean).

Figure 5.

Exposure of cultured HSNECs to IL-4 decreases expression of TLR9 protein. Th2 cytokines IL-4/IL-13 (25 $\mu\text{g}/\text{mL}$) decreased TLR9 expression in SNECs from both CRS and control patients at ALI by 50%, as determined by flow cytometry. The figure depicts a representative flow cytometry histogram plot from a single subject.

Figure 6.

Exposure of cultured HSNECs to IL-4 decreases expression of TLR9 protein, HBD-2, IL-6, and IL-8, while increasing expression of eotaxin. Cultured HSNEC were exposed to IL-4 at the ALI for 36 hours, and the supernatants were collected for analysis by ELISA. The expression of HBD-2, IL-8, and IL-6 was reduced over baseline ($p = 0.05$, 0.07 , and 0.003 , respectively), while eotaxin 3 expression was greatly increased ($p = 0.03$). Columns represent means from 10 different subjects $\pm$ standard error of the mean.

Figure 7.

Exposure of cultured HSNECs to IL-4 decreases expression of TLR9 protein and surfactant protein A mRNA. Cultured HSNEC from 10 subjects were exposed to IL-4 at the ALI for 36 hours and then processed for mRNA extraction. Real-time PCR was used to assess expression of surfactant protein A and TLR9 mRNA before and after IL-4 exposure. Expression of both innate immune genes was reduced, with TLR9 reaching statistical significance ($p = 0.01$, Wilcoxon signed rank). Columns represent means $\pm$ standard error of the mean.

Figure 8.

Role of Th2 cytokines in the characteristic pathophysiology of CRSwNP. The presence of Th2 cytokines in CRSwNP is a prominent feature that relates to the eosinophilic inflammatory histopathology. Evidence presented in this study strongly suggests that Th2 cytokines also suppress innate immune functions of SNECs, which might contribute to microbial colonization in the disease. Whether or not microbial agents in turn stimulate Th2 cytokines and eosinophilic inflammation is a topic for future study.