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Chitin stimulates expression of acidic mammalian chitinase and eotaxin-3 by human sinonasal epithelial cells *in vitro*

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Abstract

Background—Sinonasal epithelial cells participate in host defense by initiating innate immune mechanisms against potential pathogens. Antimicrobial innate mechanisms have been shown to involve Th1-like inflammatory responses. Although epithelial cells can also be induced by Th2 cytokines to express proeosinophilic mediators, no environmental agents have been identified that promote this effect.

Methods—Human sinonasal epithelial cells from patients with chronic rhinosinusitis with nasal polyps (CRSwNPs) and controls were harvested and grown in primary culture. Cell cultures were exposed to a range of concentrations of chitin for 24 hours, and mRNA for acidic mammalian chitinase (AMCase), eotaxin-3, and thymic stromal-derived lymphopoietin (TSLP) were assessed. Other cultures were exposed to interleukin 4 (IL- 4) alone and in combination with dust-mite antigen (DMA) for 36 hours. Extracted mRNA and cell culture supernatant were analyzed for expression of AMCase and eotaxin-3.

Results—Chitin induced a dose-dependent expression of AMCase and eotaxin-3 mRNA but not TSLP. Patients with recalcitrant CRSwNPs showed lower baseline expression of AMCase when compared with treatment-responsive CRSwNP and less induction of AMCase expression by chitin. DMA did not directly induce expression of AMCase or eotaxin-3. Expression of eotaxin-3 was stimulated by IL-4 and further enhanced with the addition of DMA. Levels of AMCase were not significantly affected by either IL-4 or DMA exposure. In some cases, the combination of IL-4 and DMA was able to induce AMCase expression in cell cultures not producing AMCase at baseline.

Conclusion—The abundant biopolymer chitin appears to be recognized by a yet uncharacterized receptor on sinonasal epithelial cells. Chitin stimulates production of AMCase and eotaxin-3, two pro-Th2 effector proteins. This finding suggests the existence of a novel innate immune pathway for local defense against chitin-containing organisms in the sinonasal tract. Dysregulation of this function could precipitate or exacerbate Th2 inflammation, potentially acting as an underlying factor in recalcitrant CRSwNP.

Keywords

Acidic mammalian chitinase; cell culture; chitin; eosinophils; epithelial cell; rhinitis; rhinosinusitis; sinonasal; Th2

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Chronic rhinosinusitis (CRS) is a prevalent disorder that is both complex and heterogeneous. Although multiple infectious triggers have been suggested, no common etiology has been shown to underlie CRS pathogenesis. It is generally believed that the disease results from a combination of host and environmental factors. Recent research has implicated sinonasal epithelial cells as active participants in the host immune defense of the upper airway.[1,2] Epithelial cells may be induced by both exogenous microbial products and endogenous intracellular signals to express antimicrobial effectors and proinflammatory mediators. The mechanisms through which epithelial cells initiate or potentiate inflammation may be important in the pathophysiology of CRS.

At the interface between the host and the outside world, epithelial cells play a critical role not only as a physical barrier, but also as early detectors of potential pathogens in the nasal lumen. Activation of pattern recognition receptors, such as toll-like receptors, leads to expression of multiple innate immune effector proteins.[3,4] These molecules promote defense against microbial agents, leading to a “Th1-like” local immune response.[5] In CRS with nasal polyps (CRSwNPs), the observed inflammatory response has more Th2 features, including an eosinophilic infiltrate and production of proeosinophilic mediators such as eotaxin-3 and acid mammalian chitinase (AMCase).[6,7] In CRSwNP patients, expression of toll-like receptors and innate antimicrobial effectors by epithelial cells is diminished.[8] Treatment of epithelial cells *in vitro* with the Th2 cytokines, interleukin (IL)-4 and −13, down-regulates expression of toll-like receptor 9, human β-defensin 2, IL-6, and IL-8 while up-regulating expression of eotaxin-3.[9] This finding suggests that the cytokine milieu in CRSwNP may contribute to the pattern of immune activity by epithelial cells. To date, there have been no microbial products or pathogen recognition receptors identified that result in skewing in a “Th2-like” direction. We have hypothesized that such a pathway exists and that it may be dysregulated in CRSwNP.

Sinonasal epithelial cells in normal subjects and CRS patients express AMCase, an enzyme that breaks down the molecule chitin.[7] Chitins are extremely abundant biopolymers found throughout nature, from fungal cell walls to the exoskeleton of insects.[10] In nematodes and other worms, chitin plays an important structural role in the pharynx, particularly in association with the grinder organ and the buccal cavity.[11] The existence of chitinases in mammals suggests an evolutionary role in host defense against chitin-containing organisms, such as parasites. Infection with the parasitic helminth *Nippostrongylus brasiliensis* induces AMCase expression in mice.[12] AMCase production by epithelial cells in the lung is also up-regulated by Th2 cytokines, and in severe asthma, the expression of AMCase in the human lower airway is increased.[13] We have shown abnormally elevated levels of AMCase mRNA in patients with recalcitrant CRSwNP.[7] Given what is known about the function of chitinases and their association with Th2 mucosal inflammation, chitin appears to be an attractive candidate for an exogenous molecule that triggers innate “Th2-like” inflammatory responses in the sinonasal airway.

In this study, we have exposed primary human sinonasal epithelial cells (HSNECs) derived from sinusitis patients to chitin, finding that this agent induces expression of the “pro-Th2” mediators AMCase and eotaxin-3. Also, stimulation of epithelial cells derived from control subjects with dust-mite antigen (DMA) extract shows a synergistic effect with IL-4 on stimulating eotaxin-3 expression. These results suggest that environmental interaction with chitin-containing particulates and organisms may exacerbate or even initiate eosinophilic inflammation.

METHODS

Human Subjects

Twenty-four patients undergoing transethmoid endoscopic surgery were enrolled in the study. The research protocol was approved through the Johns Hopkins Institutional Review process, and all subjects gave signed informed consent. Ten subjects had CRS with polyps failing medical management. Five of these subjects had persistent polyps >6 months after surgery and were termed “recalcitrant.” The other five had no recurrence of polyps >6 months after surgery and were termed “treatment responsive.” Fourteen additional subjects had surgery for nonsinusitis indications, including endoscopic orbital decompression, drainage of symptomatic paranasal sinus mucocele, and biopsy of sphenoid sinus masses. None of the patients met criteria for chronic sinusitis, according to the American Academy of Otolaryngology–Head and Neck Surgery Chronic Rhinosinusitis Task Force definition.[14] Sinus mucosal specimens were immediately placed in saline on ice and transported to the lab for epithelial cell isolation and culture within 2 hours of removal.

Culturing HSNECs

Primary HSNECs were isolated after procedures, as previously described.[15,16] Specimens were digested in 0.01% protease Sigma type XIV (Sigma-Aldrich, St. Louis, MO) in Ham’s F-12 media containing penicillin (100 U/mL; Invitrogen, Carlsbad, CA), amphotericin B (2.5 μg/mL; Invitrogen), and gentamicin (50 μg/mL; Invitrogen) overnight at 4°C and then neutralized by adding 10% fetal bovine serum (FBS; Invitrogen). The epithelial cells were freed by agitation and strained into a conical tube. After two washes with ice-cold PBS supplemented with 10% FBS and antibiotics, cells were seeded onto Vitrogen-coated (1:75 in sterile water; Cohesion, Palo Alto, CA) P100 dishes in bronchial epithelium growth medium (BEGM) as previously described.[17] The cells were placed overnight at 37°C and then washed with BEGM to remove debris. The cells were fed every 2 days until they reach confluence.

For differentiation, HSNECs were transferred to human type IV placental collagen (Sigma-Aldrich)-coated 6-well Falcon inserts (0.4-μm pore size; Becton Dickens, Franklin Lakes, NJ) and were grown in BEGM. When they were confluent, medium from the apical side of the culture was removed and medium below the insert was replaced with an air–liquid interface (ALI) medium consisting of LHC basal medium-DMEM-H (50:50; Invitrogen) containing the same concentrations of additives except reduced concentration of human epidermal growth factor as 0.63 ng/mL and no amphotericin B. ALI cultures were grown with no medium on the apical surface until ciliation was achieved as previously described.[18]

Stimulation of HSNEC with Chitin, IL-4, or DMA

HSNEC cultures were treated for 24 hours with 2.5–500 μg/mL of chitin derived from crab shell (Sigma). The chitin preparations used in this study have previously been shown to contain undetectable levels of endotoxin (<0.03 EU/mL), as determined by the *Limulus* amebocyte lysate assay.[19] Because chitin is not water soluble, extensive sonication was necessary to obtain suspensions at these concentrations. After incubation, supernatants were collected from apical surface and kept in −20°C until assay. In other experiments, HSNEC cultures from different subjects were exposed to IL-4 and DMA, on the apical (0.5 mL) and basolateral (2 mL) side of the cells. In separate wells containing HSNEC derived from the same subject, either 50 ng/mL of IL-4 alone or IL-4 together with 10 μg/mL of DMA (Greer Labs, Lenoir, NC) was added for 18 hours.

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 greater than 1.80 for all samples studied. Five hundred nanograms of total RNA was reverse transcribed in a 20-μL volume with random hexamer primers (Invitrogen), 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 Detection of AMCase, Eotaxin-3, and Thymic Stromal Lymphopoietin

Real-time polymerase chain reaction (PCR) was performed in Light-Cycler 1.2 (Roche Applied Science, Indianapolis, IN) using the SYBR Green PCR Kit (Qiagen). 18S (sense 5′ GTA-ACCCGTTGAACCCCATT-3′; antisense 5′-CCATCCAAT-CGGTAGTAGCG-3′) was used as an internal control. The reaction mix consisted of 50 ng of cDNA (target genes) or 5 ng of cDNA (18S RNA) and 10 μL of QuantiTect SYBR Green PCR, 0.5 mol/L of primers in total a volume of 20 μL. 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. The PCR primer sequences were as follows: AMCase sense 5′-CGC CTA CCT CAA TGT GGA TTA TGT C-3′; antisense 5′-GGT TGC TCA GGA TGA AGT TGT GTC-3′; eotaxin-3 sense 5′-AAC TCC GAA ACA ATT GTA CTC AGC TG-3′; antisense 5′-GTA-ACTCTGGGAGGAAACACCCTCTCC-3′; thymic stromal lymphopoietin (TSLP) sense 5′-CCA GGC TAT TCG GAA ACT CA-3′; and antisense 5′-CGC CAC AAT CCT TGT AAT TG-3′.

Amplicon expression in each sample was normalized to its 18S RNA content. The level of expression of target mRNA was determined as ΔCT. The ΔCT method uses the difference in CT value obtained between the normalizing housekeeping gene (18S) and the target gene to calculate relative quantification (ΔCT = the difference in threshold cycles for target and housekeeping gene). This normalization reduces sample-to-sample variations in signal strength. A decrease in the ΔCT 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.

Statistical Analysis

Raw data from ELISA and real-time PCR were entered into a spreadsheet (Excel; Microsoft Corp., Redmond, WA). Data are expressed as mean ± SEM. Statistical significance of differences was determined using the nonparametric Wilcoxon signed-rank test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Expression of AMCase and Eotaxin-3, but Not TSLP, Is Induced by Chitin in a Dose-Dependent Manner

HSNECs in culture were exposed on the apical surface with five concentrations of chitin suspended in cell culture media. After 24 hours, the cellular mRNA was extracted and analyzed by real-time PCR for expression of AMCase, eotaxin-3, and TSLP. The expression of AMCase was found to increase in a dose-dependent manner, with an average 80% induction observed at the highest chitin concentration (500 μg/mL; Fig. 1). Induction of eotaxin-3 expression was

similarly dose dependent, with a maximum average increase of 60%. Chitin stimulation caused a 20% decrease in the expression of TSLP, which did not achieve statistical significance (p = 0.07; Fig. 2).

Primary Cultures of Epithelial Cells Derived from Patients with Recalcitrant CRS Have Significantly Decreased Baseline Expression of AMCase and Eotaxin-3 and Display Increased Induction of Eotaxin-3 by Chitin

We have previously shown that whole sinonasal tissue samples from recalcitrant CRS patients have significantly greater AMCase expression than tissue from responsive CRS patients.[7] Interestingly, when grown in culture, HSNECs derived from recalcitrant CRSwNP patients show reduced baseline AMCase and eotaxin expression when compared with treatment-responsive CRS (p = 0.04 and p = 0.01, respectively; see Fig. 3). HSNECs from both groups responded to chitin with increased expression of AMCase. A marginally greater induction was observed in the treatment-responsive group than in the recalcitrant group (2.85-fold induction versus 2.07-fold induction). A 2.6-fold induction of eotaxin-3 expression was induced by chitin in the recalcitrant CRS group (p = 0.05), but there was no significant change in eotaxin-3 expression in the treatment-responsive CRS group.

Chitin Decreases Expression of TSLP by Epithelial Cells in Recalcitrant CRSwNP

Chitin decreased expression of TSLP mRNA significantly in HSNECs derived from CRSwNP patients (mean, 67%; p = 0.007; Fig. 4). A smaller and nonsignificant increase (<10%) was observed in treatment-responsive CRS epithelial cells.

Expression of Eotaxin-3 by HSNECs Is Stimulated by IL-4 and Enhanced by DMA

To model environmental exposure to chitin, HSNECs derived from 14 control subjects were exposed to DMA. DMA did not elicit expression of AMCase or eotaxin at any concentration tested. We therefore exposed sinonasal epithelial cells at the ALI to either IL-4 alone or IL-4 in combination with DMA. Analysis of the culture supernatant by ELISA after an18-hour incubation indicated that IL-4 robustly stimulated expression of eotaxin-3 (Fig. 5). The addition of DMA to the IL-4 synergistically increased the amount of eotaxin-3 present in the supernatant (p = 0.02). The average magnitude of increase in eotaxin-3 production induced by DMA was 31.5%.

Effect of IL-4, DMA, and Chitin on Expression of AMCase

Levels of AMCase mRNA in HSNECs were not significantly affected by IL-4 alone or by IL-4 and DMA in combination (Fig. 6). However, in three HNSEC cultures not producing AMCase at baseline, exposure to IL-4/DMA elicited detectable expression of AMCase mRNA.

DISCUSSION

A combination of host and environmental factors are believed to underlie the persistent sinonasal inflammation in CRS. Previous work has shown a decreased expression of innate antimicrobial genes by epithelial cells in CRSwNP, possibly contributing to microbial colonization of the sinuses. At the same time, epithelial cells in CRSwNP show increased expression of proeosinophilic mediators, which may initiate or potentiate Th2 inflammation. This imbalance of epithelial cell immune gene expression in CRSwNP can be mimicked *in vitro* by exposure of primary cultures to Th2 cytokines.[9] In this study, we showed that the naturally abundant biopolymer chitin, found in parasites, insects, and fungi, directly stimulates expression of the pro-Th2 mediators AMCase and eotaxin-3 by cultures of sinonasal epithelial cells. DMA synergistically enhances production of eotaxin-3, a potent eosinophil chemoattractant, but not AMCase. Taken together, these findings suggest a novel capacity of

epithelial cells to recognize agents potentially found in the nasal cavity that trigger an innate pro-Th2 response. Stimulation of known pattern recognition receptors on epithelial cells, such as toll-like receptors, leads to induction of "Th1-like" antimicrobial immunity. The results of this study imply that there may exist still undiscovered epithelial cell receptors with specificity for chitin and perhaps other "parasite-associated" molecules. The concept that innate immune pathways within epithelial cells can drive or promote Th2 inflammation may have important implications in the pathophysiology of CRSwNPs.

The role of chitin and chitinases in the pathophysiology of Th2-mediated inflammatory diseases such as asthma and ocular allergies has been suggested in the recent literature.[13,20] Reese *et al.* showed that chitin application to the mouse lung induces accumulation of tissue eosinophils, similar to what is observed in parasitic worm immunity.[21] Our finding that chitin stimulates AMCase and eotaxin expression by sinonasal epithelial cells supports a similar capacity to promote eosinophilic inflammation within the sinonasal tract. We have previously reported that expression of AMCase mRNA is increased in sinonasal tissue derived from patients with recalcitrant CRSwNP.[7] It is interesting that isolated epithelial cells derived from these patients do not maintain this high level of expression in culture, and, to the contrary, show a significantly reduced level of expression when compared with treatment-responsive CRS patients. One possibility is that the major source of increased AMCase mRNA in the whole sinonasal tissue samples is not epithelial cells. However, evidence from lower airway studies would argue against this, because epithelial cells appear to be the primary AMCase-producing cells in the lung. More likely, the expression of AMCase mRNA by epithelial cells *in vivo* is driven by the local milieu, which is lost when the cells are grown isolated in a culture dish. Important factors *in vivo* may include endogenous molecules such as Th2 cytokines as well as exogenous agents such as chitin-containing particulates and organisms in the nasal lumen. The significantly reduced baseline AMCase expression in cultured HSNEC highlights the concept that epithelial cell innate immune physiology is altered in recalcitrant CRS, and that this may be a pathogenic mechanism in the disease process.

While AMCase and eotaxin-3 are epithelial-derived mediators that directly induce eosinophil tissue infiltration, TSLP is a cytokine that interacts with the adaptive immune system *via* dendritic cells. Sauer *et al.* reported increased expression of TSLP in nasal polyps when compared with control sinonasal mucosa,[22] and overexpression of TSLP in a mouse model induces many features of allergic airway inflammation.[23] In addition, stimulation of airway epithelial cell lines with IL-4, IL-13, or TLR3 agonist up-regulates expression of TSLP *in vitro*.[24] Given the evidence that TSLP may play an important role in promoting Th2 airway inflammation, it is notable that chitin does not up-regulate TSLP expression. This may suggest that the signaling pathways activated by the putative chitin receptor induces transcription of a limited number of proeosinophilic effectors, without stimulating expression of other mediators associated with Th2 inflammation. Alternatively, costimulation of cytokine receptors or other pattern-recognition receptors on sinonasal epithelial cells may be necessary for chitin to induce expression of these genes. The down-regulation of TSLP in response to chitin in recalcitrant CRS epithelial cells is an unanticipated finding that invites additional investigation. Detailed cellular and molecular studies will be needed to elucidate the recognition and signal transduction molecules involved in the chitin response.

The observed responses to DMA represent a direct innate immune reaction by epithelial cells, rather than an allergic mechanism. This is consistent with the literature regarding DMA and bronchial epithelial cells. Adam *et al.* and Kauffman *et al.* both observed that human lower airway epithelial cell lines are stimulated to produce IL-8 by the DMA Der p 1.[25,26] In other studies performed by Wong *et al.*, Der p 1 was found to up-regulate expression of the adhesion molecule ICAM on bronchial epithelial cells.[27] ICAM may be important in mediating transmucosal chemotaxis by eosinophils during Th2 inflammation. Finally, Heijink *et al.*

indicated that Der p cooperated with IL-4 and TGF-β to induce expression of TARC, a pro-Th2 cytokine, by bronchial epithelial cells.[28]

The mechanisms through which DMAs induce effects on epithelial cells have not been fully elucidated. Some effects have been shown to be dependent on cysteine protease activity, while others have not. We have hypothesized that the molecule chitin, found in the exoskeletons of dust mites and other insects, may act as a pathogen-associated molecular pattern that elicits a "Th2-like" response by epithelial cells. The commercially available DMA used in our experiments is extracted from the two predominant forms of dust-mite species, *Dermatophagoides pteronyssinus* and *Dertatopagoides farinae*. Although specific allergen molecules such as Der p 1 have been identified from these species, the DMA extract contains the entire spectrum of mite proteins, including, presumably, chitin. We propose that the presence of low levels of chitin in the DMA underlies the cooperation between IL-4 and DMA in inducing eotaxin-3 expression. This concept is evidenced by the direct stimulatory effects of higher concentrations of chitin on AMCase and eotaxin-3 expression by HSNECs.

Although cell cultures imperfectly model the *in vivo* situation, the clinical relevance of chitin in Th2 airway inflammation is supported by the increased incidence of asthma among people exposed to high levels of occupational chitin, such as shellfish processors.[29] In one study, "crab asthma" was found to be present in 18% of workers in four crab processing plants in Canada. The fact that chitin is very insoluble in aqueous solutions may explain the inconsistent results we obtained with direct chitin stimulation of sinonasal epithelial cells *in vitro*. Ongoing studies seek to improve standardized delivery of chitin for challenge experiments, to begin to elucidate chitin's mechanism of action. Ultimately, the identification of a molecular pattern recognition system driving innate Th2-like immunity may lead to new therapeutic strategies in the management of recalcitrant CRSwNPs.

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References

1. Ramanathan M Jr, Lane AP. Innate immunity of the sinonasal cavity and its role in chronic rhinosinusitis. Otolaryngol Head Neck Surg 2007;136:348–356. [PubMed: 17321858]
2. Kato A, Schleimer RP. Beyond inflammation: Airway epithelial cells are at the interface of innate and adaptive immunity. Curr Opin Immunol 2007;19:711–720. [PubMed: 17928212]
3. Imler JL, Hoffmann JA. Toll receptors in innate immunity. Trends Cell Biol 2001;11:304–311. [PubMed: 11413042]
4. Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu Rev Immunol 2003;21:335–376. [PubMed: 12524386]
5. Imanishi T, Hara H, Suzuki S, et al. Cutting edge: TLR2 directly triggers Th1 effector functions. J Immunol 1 2007;178:6715–6719.
6. Olze H, Forster U, Zuberbier T, et al. Eosinophilic nasal polyps are a rich source of eotaxin, eotaxin-2 and eotaxin-3. Rhinology 2006;44:145–150. [PubMed: 16792175]
7. Ramanathan M Jr, Lee WK, Lane AP. Increased expression of acidic mammalian chitinase in chronic rhinosinusitis with nasal polyps. Am J Rhinol 2006;20:330–335. [PubMed: 16871939]
8. Lane AP, Truong-Tran QA, Schleimer RP. Altered expression of genes associated with innate immunity and inflammation in recalcitrant rhinosinusitis with polyps. Am J Rhinol 2006;20:138–144. [PubMed: 16686375]

9. Ramanathan M Jr, Lee WK, Spannhake EW, Lane AP. Th2 cytokines associated with chronic rhinosinusitis with polyps down-regulate the anti-microbial immune function of human sinonasal epithelial cells. Am J Rhinol 2008;22:115–121. [PubMed: 18416964]
10. Tharanathan RN, Kittur FS. Chitin—The undisputed biomolecule of great potential. Crit Rev Food Sci Nutr 2003;43:61–87. [PubMed: 12587986]
11. Zhang Y, Foster JM, Nelson LS, et al. The chitin synthase genes chs-1 and chs-2 are essential for C. elegans development and responsible for chitin deposition in the eggshell and pharynx, respectively. Dev Biol 2005;285:330–339. [PubMed: 16098962]
12. Nair MG, Guild KJ, Artis D. Novel effector molecules in type 2 inflammation: lessons drawn from helminth infection and allergy. J Immunol 2006;1(177):1393–1399. [PubMed: 16849442]
13. Zhu Z, Zheng T, Homer RJ, et al. Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. Science 2004;304:1678–1682. [PubMed: 15192232]
14. Meltzer EO, Hamilos DL, Hadley JA, et al. Rhinosinusitis: Establishing definitions for clinical research and patient care. Otolaryngol Head Neck Surg 2004;131(suppl 6):S1–S62. [PubMed: 15577816]
15. Ramanathan M Jr, Lee WK, Dubin MG, Lin S, et al. Sinonasal epithelial cell expression of toll-like receptor 9 is decreased in chronic rhinosinusitis with polyps. Am J Rhinol 2007;21:110–116. [PubMed: 17283572]
16. Ramanathan M Jr, Lane AP. A comparison of experimental methods in molecular chronic rhinosinusitis research. Am J Rhinol 2007;21:373–377. [PubMed: 17621826]
17. Saatian B, Yu XY, Lane AP, et al. Expression of genes for B7–H3 and other T cell ligands by nasal epithelial cells during differentiation and activation. Am J Physiol Lung Cell Mol Physiol 2004;287:L217–L225. [PubMed: 15047568]
18. Lane AP, Saatian B, Yu XY, Spannhake EW. mRNA for genes associated with antigen presentation are expressed by human middle meatal epithelial cells in culture. Laryngoscope 2004;114:1827–1832. [PubMed: 15454780]
19. Shibata Y, Foster LA, Bradfield JF, Myrvik QN. Oral administration of chitin down-regulates serum IgE levels and lung eosinophilia in the allergic mouse. J Immunol 2000;164:1314–1321. [PubMed: 10640745]
20. Musumeci M, Bellin M, Maltese A, et al. Chitinase levels in the tears of subjects with ocular allergies. Cornea 2008;27:168–173. [PubMed: 18216571]
21. Reese TA, Liang HE, Tager AM, et al. Chitin induces accumulation in tissue of innate immune cells associated with allergy. Nature 2007;447:92–96. [PubMed: 17450126]
22. Sauer J, Damm M, Rosenbohm J, et al. Gene expression of thymic stromal lymphopoietin in nasal mucosa and nasal polyps. Otolaryngol Head Neck Surg 2004;131:190.
23. Zhou B, Comeau MR, De Smedt T, et al. Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. Nat Immunol 2005;6:1047–1053. [PubMed: 16142237]
24. Kato A, Favoreto S Jr, Avila PC, Schleimer RP. TLR3-and Th2 cytokine-dependent production of thymic stromal lymphopoietin in human airway epithelial cells. J Immunol 2007;179:1080–1087. [PubMed: 17617600]
25. Adam E, Hansen KK, Astudillo Fernandez O, et al. The house dust mite allergen Der p 1, unlike Der p 3, stimulates the expression of interleukin-8 in human airway epithelial cells via a proteinase-activated receptor-2-independent mechanism. J Biol Chem 2006;281:6910–6923. [PubMed: 16293628]
26. Kauffman HF, Tamm M, Timmerman JA, Borger P. House dust mite major allergens Der p 1 and Der p 5 activate human airway-derived epithelial cells by protease-dependent and protease-independent mechanisms. Clin Mol Allergy 2006;4:5. [PubMed: 16569217]
27. Wong CK, Li ML, Wang CB, et al. House dust mite allergen Der p 1 elevates the release of inflammatory cytokines and expression of adhesion molecules in co-culture of human eosinophils and bronchial epithelial cells. Int Immunol 2006;18:1327–1335. [PubMed: 16798840]
28. Heijink IH, Marcel Kies P, van Oosterhout AJ, et al. Der p, IL-4, and TGF-beta cooperatively induce EGFR-dependent TARC expression in airway epithelium. Am J Respir Cell Mol Biol 2007;36:351–359. [PubMed: 17023689]

29. Cartier A, Malo JL, Forest F, et al. Occupational asthma in snow crab-processing workers. J Allergy Clin Immunol 1984;74:261–269. [PubMed: 6470360]

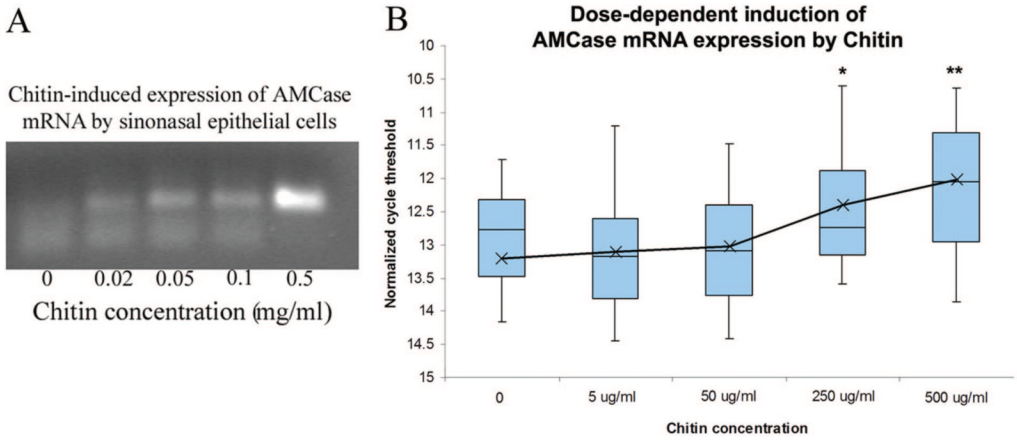

Figure 1.

Exposure of human sinonasal epithelial cells in primary culture to chitin causes a dose-dependent expression of acidic mammalian chitinase (AMCase). (A) Reverse-transcription polymerase chain reaction (PCR) shows increasing expression of AMCase mRNA by sinonasal epithelial cells in response to chitin. (B) Real-time PCR dose–response curve showing AMCase mRNA expression as a function of chitin concentration (n = 10 chronic rhinosinusitis subjects; *p = 0.048 and **p = 0.013 versus control, Wilcoxon signed-rank test).

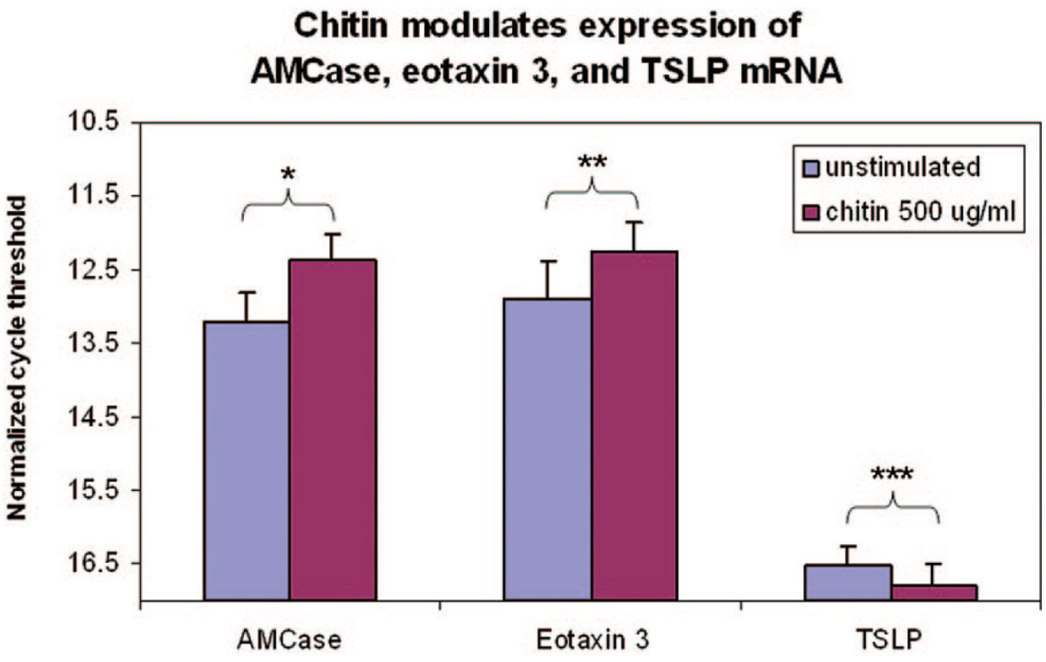

Figure 2.

Real-time polymerase chain reaction analysis of expression of acidic mammalian chitinase (AMCase), eotaxin-3, and thymic stromal-derived lymphopoietin (TSLP) by human sinonasal epithelial cells after exposure to 500 μg/mL chitin. Levels of AMCase and eotaxin-3 mRNA were significantly increased by a mean of 80 and 60%, respectively. Expression of TSLP decreased 30% (n = 10 chronic rhinosinusitis patients; *p = 0.01, **p = 0.02, and ***p = 0.07, paired two-sample t-test).

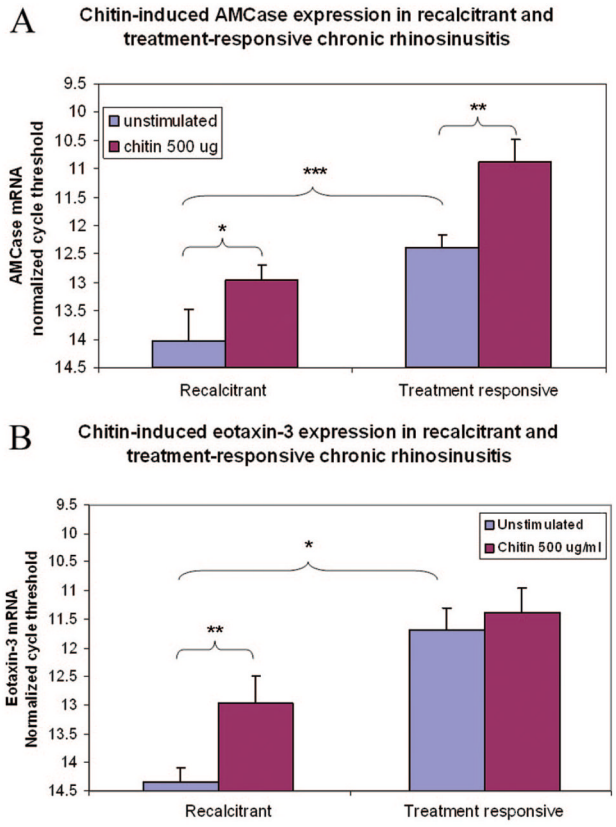

Figure 3.

Baseline and chitin-induced expression of acidic mammalian chitinase (AMCase) and eotaxin-3 by sinonasal epithelial cells in chronic rhinosinusitis (CRS) differs as a function of disease responsiveness to therapy. (A) Baseline AMCase expression is decreased in recalcitrant CRS with nasal polyps patients when compared with treatment-responsive patients. Chitin induces expression of AMCase mRNA in both groups (n = 5 subjects per group; *p = 0.04, **p = 0.03, and ***p = 0.02, paired two sample t-test). (B) Baseline epithelial cell eotaxin-3 mRNA expression is increased in treatment responsive CRS when compared with recalcitrant subjects. Chitin induces increased expression of eotaxin-3 mRNA in the recalcitrant group, but not in the treatment-responsive group (n = 5 subjects per group; *p = 0.003, two sample t-test assuming unequal variances and **p = 0.05, paired two sample t-test).

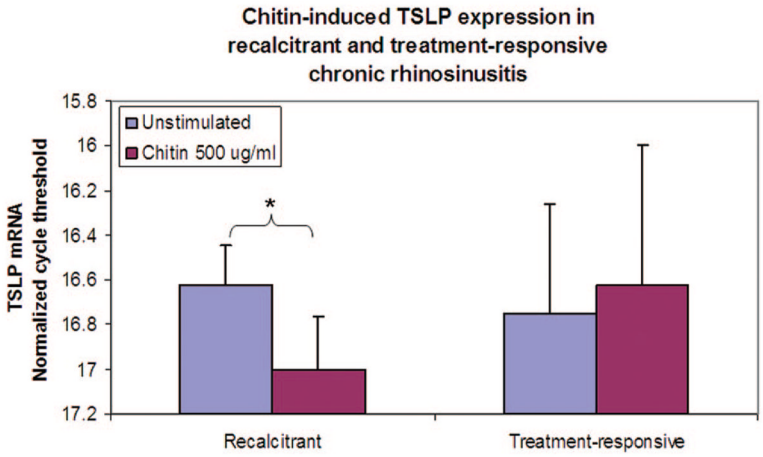

Figure 4.

Expression of thymic stromal lymphopoietin (TSLP) is significantly down-regulated by chitin in sinonasal epithelial cells derived from recalcitrant chronic rhinosinusitis with nasal polyps (CRS) patients but is unchanged in cells from treatment-responsive patients. The baseline level of TSLP mRNA is similar in both groups (n = 5 CRS subjects per group; *p = 0.007, paired two-sample t-test).

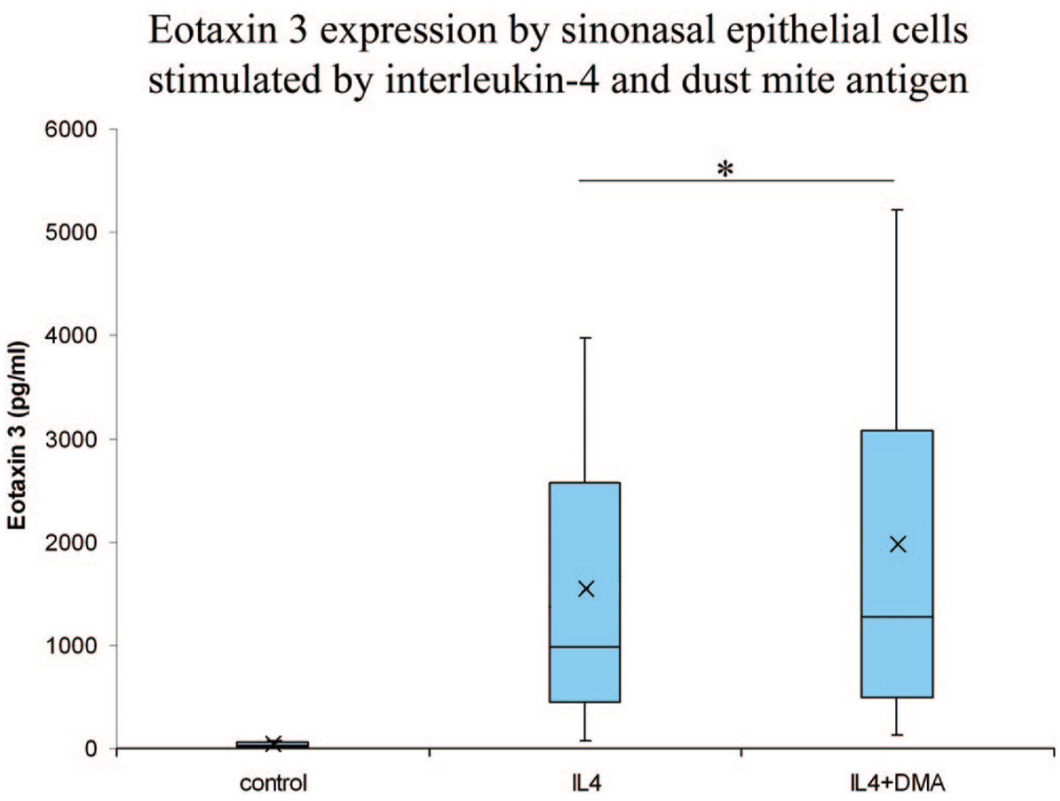

Figure 5.

Expression of eotaxin-3 by sinonasal epithelial cells is potently stimulated by interleukin (IL)-4 and further enhanced by dust-mite antigen (DMA). Differentiated epithelial cells from control subjects without chronic rhinosinusitis were exposed for 18 hours to either IL-4 alone or IL-4 with DMA. The concentration of eotaxin-3 in the culture media supernatant was assessed by ELISA (n = 14 control subjects; *p = 0.03, Wilcoxon signed-rank test).

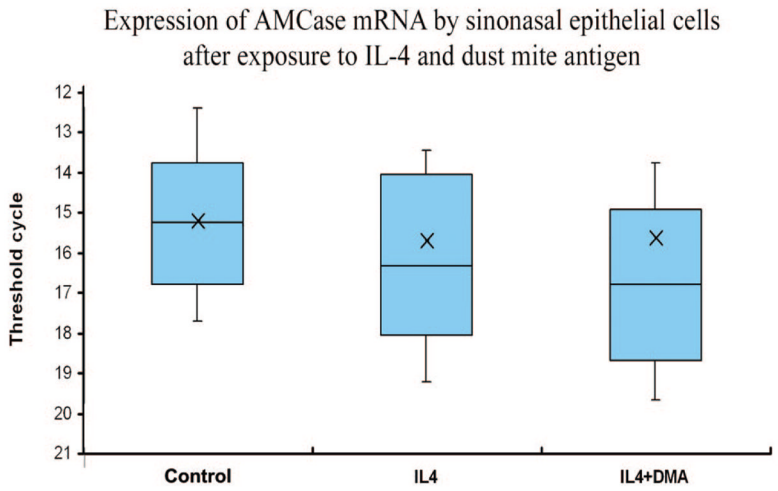

Figure 6.

Expression of acidic mammalian chitinase (AMCase) mRNA by sinonasal epithelial cells was unchanged after exposure for 18 hours to either interleukin (IL)-4 alone or IL-4 with dust-mite antigen. The level of AMCase mRNA expression was measured by real-time polymerase chain reaction (n = 14 control subjects).