

Drivers of chronic rhinosinusitis: Inflammation versus infection

Daniel L. Hamilos, MD *Boston, Mass*

Studies of the underlying cause or causes of chronic rhinosinusitis (CRS) over the past 20 or more years have expanded from a focus on systemic immune and allergic mechanisms to an intense search for the underlying drivers of mucosal inflammation. These drivers involve mucosal inflammatory pathways that become activated by allergens, microbial stimuli, or poorly understood exogenous or endogenous stimuli. The holy grail in the study of CRS is to identify specific drivers of mucosal inflammation and translate these into more effective treatment for CRS. Certain deficiencies in local innate immunity have been described in patients with CRS that predispose to increased sinus mucosal bacterial colonization/infection, including deficient local production of antimicrobial lactoferrin and deficient functioning of the bitter taste receptor TAS2R38. Conversely, certain innate factors, namely IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), are elaborated by sinus epithelial cells in response to microbial stimulation or airway injury and promote local T_H2 inflammation. The precise physiologic role of these factors in innate or adaptive immunity is unclear, although IL-33 might function as an alarmin triggered by damage-associated molecular patterns. The cytokines IL-25 and TSLP, similarly promote proinflammatory tissue responses. Another feature of epithelial dysregulation in patients with CRS is overproduction of eosinophil-promoting C-C chemokines by sinus epithelium, perhaps driven in part through innate stimuli, as well as T_H2 cytokines, such as IL-13. Strategies to reduce the microbial stimulation of maladaptive T_H2 inflammation or to suppress the local elaboration of T_H2-promoting epithelial factors, such as IL-33, have potential therapeutic benefit in patients with CRS, although the extent to which this is realized in patient care remains limited at present. This rostrum will summarize my views on the major microbial drivers of mucosal inflammation and dysregulation of innate T_H2-promoting factors in patients with CRS based on recent experimental data. (*J Allergy Clin Immunol* 2015;136:1454-9.)

Key words: *Chronic rhinosinusitis, nasal polyposis, T_H2 inflammation, innate, adaptive*

Abbreviations used

CRS: Chronic rhinosinusitis
 CRSsNP: Chronic rhinosinusitis without nasal polyps
 CRSwNP: Chronic rhinosinusitis with nasal polyps
 DAMP: Damage-associated molecular pattern
 NP: Nasal polyp
 PNEC: Primary nasal epithelial cell
 SEB: Staphylococcal enterotoxin B
 TLR: Toll-like receptor
 TSLP: Thymic stromal lymphopoietin

The heterogeneity of chronic rhinosinusitis (CRS) is being acknowledged more and more, going well beyond the clinical classification system of chronic rhinosinusitis without nasal polyps (CRSsNP), chronic rhinosinusitis with nasal polyps (CRSwNP), and allergic fungal rhinosinusitis¹ to an emphasis on defining CRS endotypes (ie, subclassifying CRS into subsets based on distinct functional or pathobiological mechanisms of disease).² The hope is that endotyping CRS will lead to more precise strategies for specific therapeutic interventions, such as use of anti-inflammatory cytokine modulators or antibiotics.

Studies of the underlying cause or causes of CRS over the past 20 or more years have expanded from a focus on systemic immune or allergic mechanisms to an intense search for the underlying drivers of mucosal inflammation. That is not to say that systemic immune or allergic mechanisms are not important drivers of inflammation but rather that these mechanisms alone are insufficient to explain the intense and persistent inflammation seen in patients with CRS (Figs 1 and 2). The vast majority of patients with CRS do not have systemic immunodeficiency. Furthermore, systemic allergy does not inevitably lead to CRS and might instead lead only to allergic rhinitis.

We now appreciate that other sinus mucosal inflammatory cytokine pathways are involved in persistent inflammation in patients with CRS. These pathways are broadly categorized as those involved in innate immunity, noninfectious damage-associated molecular patterns (DAMPs), or alarmins and other cytokines that induce proinflammatory tissue responses. Examples of the innate immune factors include local elaboration of antimicrobial peptides (eg, lactoferrin, β -defensin, or cathelicidin) or innate pattern recognition receptors, such as Toll-like receptors (TLRs), NOD receptors, or bitter taste receptors. A deficiency in 1 or more of these factors might drive infectious inflammation in patients with CRS.

A review of deficiencies in antimicrobial peptides and innate pattern recognition receptors associated with CRS was recently

From the Division of Rheumatology, Allergy and Immunology, Massachusetts General Hospital.

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Corresponding author: Daniel L. Hamilos, MD, Division of Rheumatology, Allergy and Immunology, Massachusetts General Hospital, Bullfinch 422, 55 Fruit St, Boston, MA 02114. E-mail: dhamilos@partners.org.

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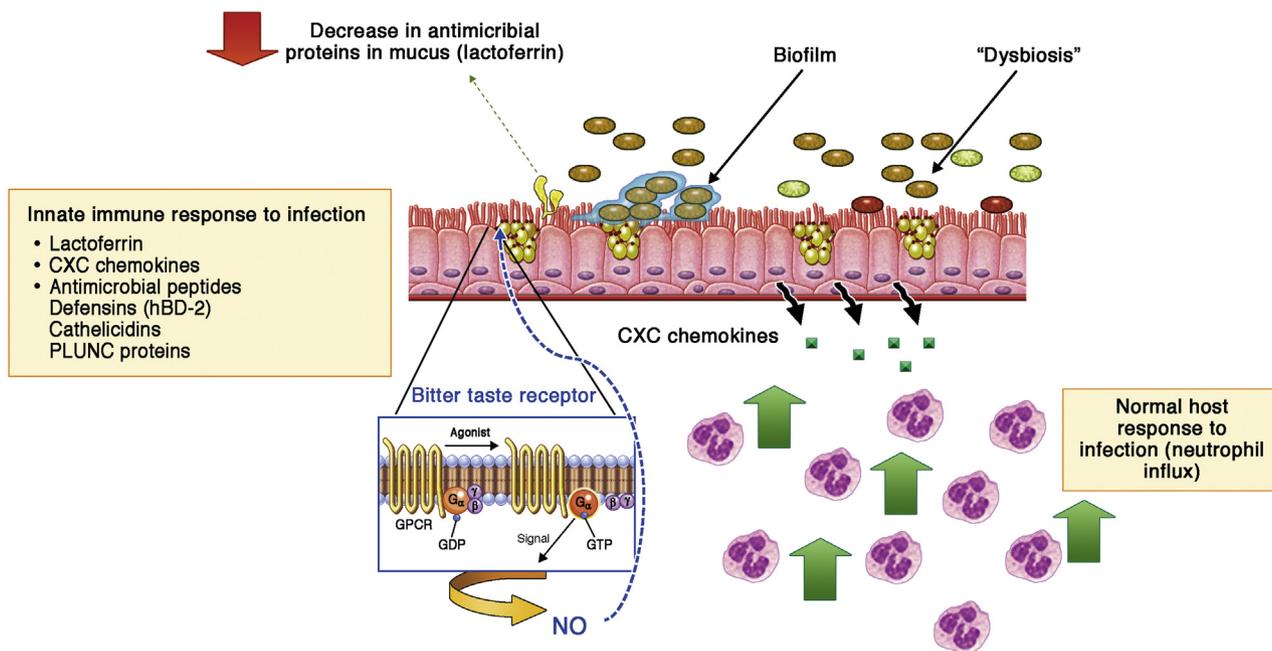


FIG 1. Drivers of “infectious” inflammation in patients with CRS. There is evidence for an association between bacterial biofilm and “dysbiosis” of the normal sinus microbiome and persistent infectious (neutrophilic) inflammation in patients with CRS. To date, the best described deficiencies in innate immunity associated with CRS are a deficiency in mucosal lactoferrin production^{3,4} and a genetic polymorphism in the bitter taste receptor gene *TAS2R38*,⁵ which results in a loss-of-function receptor leading to an inability to sense a quorum-sensing molecule elaborated by *Pseudomonas aeruginosa* and generate normal antimicrobial nitric oxide (NO) levels. In contrast, no primary defects in other epithelial pattern recognition receptors (eg, TLRs or NOD receptors) have been described in patients with CRS. Antimicrobial peptides produced in epithelial cells include (among others) lactoferrin, defensins (human b-defensin 2), cathelicidins, and PLUNC proteins. AC, Adenylate cyclase. The bitter taste receptor is a G-protein coupled receptor.

published.¹⁶ To date, the best described innate deficiencies are a deficiency in mucosal lactoferrin production^{3,4} and a genetic polymorphism in the bitter taste receptor gene *TAS2R38*,⁵ which results in a loss-of-function receptor leading to an inability to sense a quorum-sensing molecule elaborated by *Pseudomonas aeruginosa* and generate normal antimicrobial levels of nitric oxide. In contrast, no primary defects in epithelial TLRs or NOD receptors have been described in patients with CRS.

A variety of DAMPs or alarmins have been described, including (among others) heat shock proteins, hyaluronan, galectins, thioredoxin, adenosine, high-mobility group box protein 1, IL-1 α , and IL-33.¹⁷ In general, it is the overproduction of DAMPs, such as IL-33, that has been implicated in patients with chronic inflammatory diseases, including CRS.¹⁸⁻²¹ Overproduction of other cytokines not categorized as DAMPs has also been associated with persistent tissue inflammation. Examples include the elaboration of IL-25 or thymic stromal lymphopoietin (TSLP), which promote eosinophilic inflammation in patients with nasal polyposis,¹⁹⁻²⁴ or overproduction of C-C chemokines by sinus epithelial cells, such as RANTES (CCL5), monocyte chemoattractant protein 2 (CCL8), monocyte chemoattract protein 3 (CCL7) and eotaxin (CCL11) that chemoattract lymphocytes, monocytes, and eosinophils into sinus mucosa, which also occurs most dramatically in patients with nasal polyposis.²⁵ It remains unclear whether elaboration of DAMPs or other proinflammatory factors serves a protective

innate immune function and whether strategies to suppress their local elaboration offer potential therapeutic benefit.²⁶

Therefore the key question is as follows: “What drives chronic inflammation in patients with CRS”? This brief review will summarize my opinions on this question based on recent experimental data.

IS BIOFILM A DRIVER OF INFLAMMATION IN PATIENTS WITH CRS?

First, we must ask why biofilm occurs on mucosal surfaces, such as sinus epithelium, in patients with CRS. In experimental rabbits and sheep^{27,28} biofilm can be a consequence of sinus ostial blockage and subsequent sinus bacterial infection. In the clinical setting defects in mucociliary clearance or epithelial barrier function or an underlying defect in innate immunity serve to promote local infection and biofilm formation beyond that afforded by sinus ostial blockage alone. Less obvious is the fact that maladaptive T_H2 inflammation has been shown to induce secondary defects in innate immunity, including downregulation of TLR9 expression in cultured sinonasal epithelial cells *in vitro* that might serve to promote infection.²⁹

There is an abnormal presence of bacteria in patients with CRS, as evidenced by the finding of bacterial biofilm in 56% of refractory surgical cases and the association between the finding of bacterial biofilm and worse outcomes after endoscopic sinus

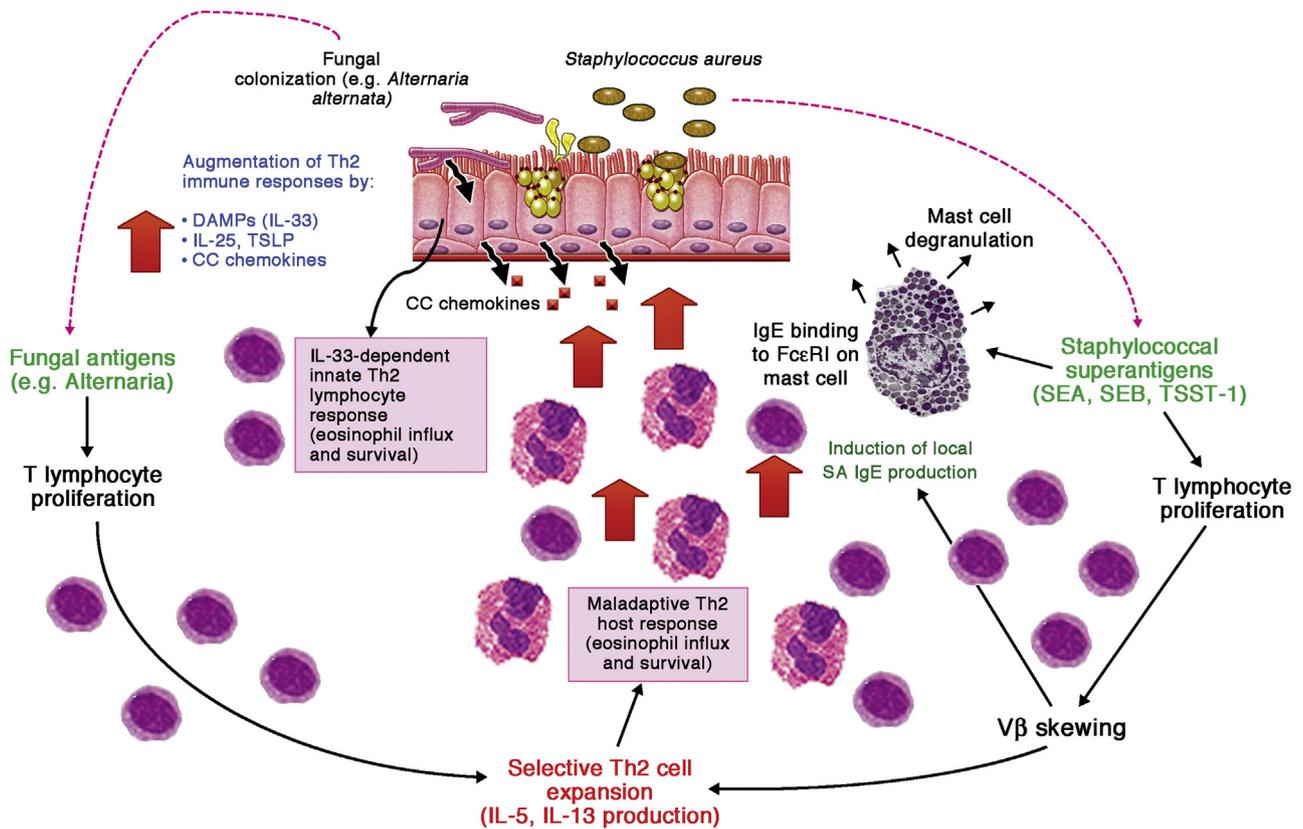


FIG 2. Drivers of maladaptive T_H2 inflammation and augmentation of T_H2 immune responses by innate inflammatory cytokines from dysregulated epithelial cells in patients with CRSwNP. The local tissue inflammatory response in patients with CRSwNP is strongly biased toward eosinophilic T_H2 inflammation, often despite a lack of systemic evidence for allergic disease.⁶ *Staphylococcus aureus* can serve as a microbial driver for maladaptive T_H2 inflammation in patients with CRSwNP by virtue of staphylococcal superantigen-induced local T-lymphocyte stimulation and production of superantigen-specific IgE.⁷⁻¹¹ T lymphocytes in NPs are skewed toward enrichment for $V\beta$ elements that are known to respond to staphylococcal superantigens,¹⁰⁻¹² and SEB induces robust production of IL-5 and IL-13 in dispersed NP T lymphocytes.¹³ Certain fungi, particularly *Alternaria* and *Candida* species, induce production of IL-5 and IL-13, as well as IFN- γ , in peripheral blood lymphocytes from patients with CRS and might therefore also serve as a driver for the local T_H2 inflammatory response.¹⁴ *A alternata* might also drive innate T_H2 inflammation mediated through IL-33 and innate T_H2 lymphocytes.¹⁵ Dysregulated epithelial proinflammatory factor production, including overproduction of IL-25, TSLP, and C-C chemokines, also serves to drive T_H2 inflammation in patients with CRSwNP independent of T_H2 adaptive immunity. The underlying drivers of these T_H2 proinflammatory signals in patients with CRSwNP remain unclear. Adapted from Hamilos.¹⁶

surgery. Only recently have biofilm studies pointed to a connection between innate immunity and the propensity toward biofilm formation. Psaltis et al³ reported a decrease in lactoferrin expression at both the mRNA and protein levels in patients with CRS and an even further decrease in expression in the presence of bacterial biofilm. A study by Lee et al⁵ identified bitter taste receptors in the sinus mucosa and a polymorphism in the bitter taste receptor T2R38 that confers inability to recognize a quorum-sensing molecule from *P aeruginosa*. Normally, activation of this receptor results in production of nitric oxide. Presence of the nonfunctional *TAS2R38* receptor phenotype has been shown to correlate with increased risk for *P aeruginosa* infection in patients with refractory CRS. These findings are likely just the tip of the iceberg in terms of our knowledge about host microbial interactions that control bacterial colonization and infection in the sinus mucosa.¹⁶

Epithelial barrier function is important for maintaining mucosal hydration and preventing penetration of foreign

particles, including microbes, into the subepithelial layer. Defective epithelial barrier function has been found to be a key risk factor for development of atopic dermatitis, leading to increased transepidermal water loss and possibly contributing directly to increased susceptibility to *Staphylococcus aureus* skin infection.³⁰ To date, no primary defects in epithelial tight junction proteins have been described in patients with CRS or nasal polyps (NPs). Soyka et al³¹ found that NPs have decreased transtissue resistance and an irregular, patchy decreased expression of the tight junction proteins occludin and zonula occludens 1, but these effects are likely secondary to tissue inflammation because it was shown that *in vitro* culture of healthy control or NP epithelial cells in the presence of IL-4 resulted in downregulation of occludin and zonula occludens 1 expression, whereas culturing with IFN- γ caused an upregulation of these proteins.

Mucociliary clearance is an essential process in normal sinus function. Impairments in mucociliary clearance lead to mucostasis, bacterial colonization, biofilm formation, and CRS

in patients with primary ciliary dyskinesia. Mutational analyses have discovered that 38% of patients with primary ciliary dyskinesia carry mutations of the dynein genes *DNAI1* and *DNAH5*.³² However, the vast majority of patients with CRS do not carry mutations in genes known to be involved in ciliary motility, and genetic association studies of patients with refractory CRS have not uncovered any gene polymorphisms that would suggest a primary defect in mucociliary clearance.³³

More studies are needed on whether additional underlying defects in innate immunity predispose to biofilm formation. For example, a recent study found gene expression differences in patients with biofilm-positive versus biofilm-negative CRS, including the following genes involved in regulation of nitric oxide or reactive oxygen species generation against *S aureus*: *OXR1*, *PRDX6*, *NCF2*, and *PRNP*.³⁴ Whether any of these genes result in defective innate immunity is yet to be determined. An experimental model using explants of NP tissue might also provide greater insight into the effects of *S aureus* biofilm.³⁵

IS DYSBIOSIS OF THE SINUS MICROBIOME A DRIVER OF INFLAMMATION IN PATIENTS WITH CRS?

Studies of the microbiome in healthy versus diseased sinus mucosa have fairly consistently reported that the burden of bacterial organisms in the sinus mucosa is not significantly altered in patients with CRS but that the bacterial diversity (richness and evenness) is reduced, whereas the burden of certain bacteria, most notably *S aureus*, is increased (as reviewed by Wilson and Hamilos³⁶). Most studies have included both patients with CRSsNP and patients with CRSwNP, and earlier culture studies also suggest that the abundance of *S aureus* is increased in patients with CRSwNP.³⁷ Another intriguing finding in one study of the CRS microbiome was the observation that in comparison with the normal healthy sinus, the CRS microbiome is characterized by an overrepresentation of certain harmful bacterial taxa or species (eg, *Corynebacterium tuberculostrictum*) and underrepresentation of other protective bacteria, most notably lactobacilli (eg, *Lactobacillus sakei*).³⁸ Studies such as this provide some hope that strategies to correct the dysbiosis in the CRS microbiome might lead to improved sinus health.

ARE SPECIFIC BACTERIA DRIVERS OF MALADAPTIVE T_H2 INFLAMMATION IN PATIENTS WITH CRS?

Here the answer is definitely yes. It has long been recognized that CRSwNP is a disease in which the local tissue inflammatory response is typically strongly biased toward T_H2 inflammation, often despite a lack of systemic evidence for allergic disease.⁶ To a lesser extent, patients with CRSsNP also manifest tissue eosinophilia.^{39,40} There is evidence that links colonizing microorganisms to this local maladaptive T_H2-type inflammation in patients with CRSwNP.

In regard to the observed increased abundance of *S aureus* in the CRS microbiome, it has been established that *S aureus* can serve as a microbial driver for maladaptive T_H2 inflammation in patients with CRSwNP by virtue of staphylococcal superantigen-induced local T-lymphocyte stimulation and production of superantigen-specific IgE.⁷⁻¹¹ Mucosal colonization with *S aureus* has been found in 64% of patients with CRSwNP compared with roughly

30% of healthy subjects or patients with CRSsNP.³⁷ In a study of 13 patients with massive polyposis, 55% of patients were found to have enterotoxin-producing *S aureus* in the nasal mucus adjacent to polyps.¹¹ It was further shown that T lymphocytes isolated from the polyps showed a skewing of V β use with enrichment for V β s known to respond to staphylococcal superantigens.¹⁰⁻¹² Finally, staphylococcal enterotoxin B (SEB) was found to induce robust production of IL-5 and IL-13 in dispersed NP T lymphocytes.¹³ These studies suggest that colonizing *S aureus* might be a major driver of the local maladaptive T_H2 inflammatory response in patients with CRSwNP.

ARE FUNGI A DRIVER OF MALADAPTIVE T_H2 INFLAMMATION IN PATIENTS WITH CRS?

Fungi are commonly detected in the attached mucus of sinus tissues in patients with CRS and can induce eosinophil activation and degranulation.⁴⁰ Certain fungi, particularly *Alternaria* and *Candida* species, were shown to induce production of IL-5 and IL-13, as well as IFN- γ , in peripheral blood lymphocytes from patients with CRS. It was hypothesized that this would account for a maladaptive mixed T_H1/T_H2 eosinophil-promoting mucosal immune response.¹⁴ However, a recent study did not demonstrate consistent IL-5 production in response to *Alternaria* species in patients with CRS from Utah.⁴¹ Furthermore, relative to *S aureus* enterotoxin SEB, fungal allergens elicit modest production of IL-5 and IL-13 from dispersed NP T lymphocytes.¹³ It remains unclear whether there is a subset of patients with CRS that is highly susceptible to mucosal T_H1/T_H2 maladaptive inflammation, although clinically, some patients with refractory CRS respond dramatically to oral itraconazole administered in conjunction with topical budesonide sinus rinses. Recent studies suggest that *Alternaria alternata* can drive innate T_H2 inflammation mediated through IL-33 (see below).

DOWNREGULATION OF EPITHELIAL INNATE IMMUNITY BY MALADAPTIVE T_H2 TISSUE INFLAMMATION

Given the strong maladaptive T_H2-type chronic inflammation characteristic of patients with CRSwNP, investigations were undertaken to ascertain whether T_H2-type inflammation modulated innate immune function. Ramanathan et al²⁹ found that TLR9 expression on cultured primary nasal epithelial cells (PNECs) from patients with CRSwNP was reduced by 50% compared with that on control PNECs. Culturing control PNECs in the presence of the T_H1 cytokine IFN- γ increased TLR9 expression by 49%, whereas culturing in the presence of the T_H2 cytokines IL-4 or IL-13 decreased TLR9 expression by 46.6%, suggesting that T_H2 tissue inflammation downregulates epithelial innate immunity. This illustrates the complex interplay between host adaptive immune responses and local innate immunity promoting T_H2-type inflammation.

ARE DYSREGULATED EPITHELIAL PROINFLAMMATORY FACTORS DRIVERS OF INNATE T_H2 OR MALADAPTIVE T_H2 INFLAMMATION IN PATIENTS WITH CRS?

The cytokines IL-25, IL-33, and TSLP are produced and released by airway epithelial cells in response to various

environmental and microbial stimuli or by cellular damage.^{42,43} IL-25 and IL-33 induce T_H2 cytokine production by innate lymphoid cells and IL-13 production by macrophages.⁴² In an experimental mouse model, systemic administration of IL-33 promoted airway eosinophilia, as well as increased production of T_H2-type cytokines and mucus, in the lungs. These effects were independent of adaptive immune response and mediated by type 2 innate lymphoid cells. Furthermore, *in vivo* airway administration of *A alternata* induced T_H2-type airway inflammation that was dependent on IL-33 and type 2 innate lymphoid cells independent of T_H2 adaptive immunity.¹⁵ IL-33 appears to be involved in the early innate T_H2 response and might not be necessary for subsequent adaptive immune responses.⁴² In mouse models of asthma, TSLP also promotes airway T_H2 inflammation, including airway eosinophilia, increases in serum IgE and bronchoalveolar lavage T_H2 cytokine levels, airway hyperresponsiveness, and increased mucus production.⁴⁴ These effects appear to be primarily through amplification of adaptive T_H2 responses rather than innate T_H2-type immunity.⁴² Taken together, IL-25, IL-33, and TSLP represent a novel array of epithelial proinflammatory cytokines that promote innate T_H2 or maladaptive T_H2 inflammation in the airways, including the sinus epithelium. IL-33 and TSLP levels are increased in NPs,^{21,23} and their potential role in promoting T_H2 inflammation in an IgE-independent manner is intriguing, given the clinical observation that many patients with CRSwNP are nonallergic.⁴⁵

SYNTHESIS

Many cases of CRS, including both the CRSsNP and CRSwNP subtypes, as well as allergic fungal rhinosinusitis, remain challenging to treat, with limited benefit from either surgical and medical management. Furthermore, patients with CRS showing signs of mucosal infection despite surgery to reestablish sinus patency are often the most difficult to manage, and the following question often arises: "How much of this disease is driven by infection, and how much is driven by inflammation?" At present, we do not have simple clinical tools to precisely answer this question, and the clinician is limited to using data from sinus tissue pathology reports, sinus secretions, and bacterial and fungal sinus cultures to guide treatment. Hopefully, this situation will improve soon with application of more precise tools, such as measurement of cytokines in sinus secretions or sinus tissue biopsy specimens and molecular techniques to identify and quantify infection or the sinus microbiome. Techniques such as these should help define more precise CRS endotypes and guide therapy. Although we do not have specific antibiotic strategies that eradicate bacterial biofilm, it is my impression that biofilm can be eradicated or at least controlled in most patients after sinus surgery with use of antibiotics to eradicate obvious infection and topical sinus rinses.⁴⁵ Topical steroid sinus instillations are especially helpful in patients with recurrent polypoid disease. There might also be a role for systemic antifungal drugs, such as itraconazole, in some patients whose symptoms have not improved despite eradicating obvious bacterial infections and using topical steroid instillations, although it remains challenging to identify such patients without empirically trying itraconazole for 1 to 3 months.⁴⁶ Strategies to downregulate maladaptive T_H2 inflammation, such as use of cytokine-blocking mAbs, offer promise but have only received limited study for the treatment of CRSwNP. Strategies to

downregulate dysregulated epithelial proinflammatory factors, such as IL-25, IL-33, and TSLP, which drive innate T_H2 inflammation in patients with CRS, have yet to be studied at all in the setting of CRS. Clearly, we have much to learn, and many of our patients are desperate for progress in this area.

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