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Host-microbial interactions in patients with chronic rhinosinusitis

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Activity Objectives

1. To know that the presence of bacterial biofilm in sinus tissues of patients with CRS is associated with a worse prognosis after sinus surgery.
2. To know that the defect in innate immunity most commonly found in patients with refractory CRS is a decrease in lactoferrin levels in sinus secretions.
3. To know that disturbances in mucociliary clearance in patients with CRS are reversible with clearance of infection and re-establishment of sinus ostial patency.
4. To understand that colonization with either fungi or *Staphylococcus aureus* has been associated with "maladaptive" T_H2-type immune responses that contribute to persistent inflammation in patients with refractory CRS.
5. To know that the bacterial species with an increase in abundance ("bacterial burden") in patients with refractory CRS is *S aureus.*
6. To know that the fungal species that is increased in abundance ("fungal burden") in patients with refractory CRS is *Alternaria* species.
7. To understand that maladaptive T_H2 inflammation in the sinuses might negatively affect innate immunity in sinus mucosa by down-regulating TLR9 expression.

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CLINICAL VIGNETTE

A 59-year-old white woman presented for evaluation of recurrent nasal polyposis. She first had nasal polyps removed at age 34 years, with subsequent recurrences and 4 additional functional endoscopic sinus surgeries, most recently 6 weeks ago. Before her last surgery, she had pain in the right side of her nose, total nasal blockage, decreased sense of smell, a smell of "something rotting," postnasal drainage, discomfort in the lower right jaw, and perfuse watering of the right eye. She has occasional shooting pains in the right cheek or numbness of the right cheek and pain above the right eyebrow.

During the 3 months before her last sinus surgery, she had taken antibiotics 4 times, including azithromycin and erythromycin with a methylprednisolone dose pack. She had used intranasal fluticasone for 10 years. Cetirizine and montelukast were started 3 weeks before her evaluation.

The patient had a history of asthma for which she used albuterol 5 d/wk. Her nasal symptoms were year round, without seasonal worsening. Allergy skin testing 1 week earlier revealed positive results to a few pollens, dust mites, cat dander, *Aspergillus* species, and *Penicillium* species. Lung function was normal.

Her environmental history was unremarkable, with no pet exposure, mold, mildew, or dampness in the home. She had a remote 13 pack-year history of smoking. She is an artist and painter who uses acrylic paints. Her family history and past medical history were unremarkable. Her current medications

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included omeprazole, paroxetine, montelukast, cetirizine, triazolam, and dilute budesonide nasal rinses.

On physical examination, she appeared healthy, with normal vital signs and normal examination results, except for her nose. Her nose revealed postoperative changes bilaterally without polyps but moderate thick, bright-green mucus in the right anterior ethmoid region.

Her laboratory data included a normal complete blood cell count and white blood cell differential, an erythrocyte sedimentation rate of 43, normal serum protein electrophoresis, an IgG level of 1460, an IgA level of 168, an IgM level of 481 (increased), and an IgE level of 2203 (increased). The result of an IgE CAP RAST test for *Aspergillus fumigatus* was markedly increased at 41.2 kU/L (normal <0.35 kU/L). Her preoperative head computed tomographic scan revealed no intracranial abnormalities but extensive mucosal thickening in the right maxillary sinus, right ethmoid air cells, and right sphenoid (see Fig E1 in this article's Online Repository at www.jacionline.org). The frontal sinuses and left-side sinuses were clear.

Functional endoscopic sinus surgery was performed. The intraoperative report indicated the presence of extensive polyps and thick mucus described as "peanut butter" or "allergic mucin." A pathologic specimen from the right sinus revealed polypoid chronic sinusitis with stromal eosinophilia. The mucus sample revealed "mucoid debris with mixed neutrophilic and eosinophilic inflammation." A Grocott methenamine silver and periodic acid-Schiff diastase stain of the mucus was negative for fungi. The mucin grew a few *Staphylococcus aureus* sensitive to oxacillin and a few *Pseudomonas aeruginosa*. Results of a fungal culture were negative.

The full version of this article, including a review of relevant issues to be considered, can be found online at www.jacionline.org. If you wish to receive CME or MOC credit for the article, please see the instructions above.

INTRODUCTION

Chronic rhinosinusitis (CRS) remains an enigmatic inflammatory disease. It often arises in patients who were previously healthy. Although it is classified into subsets of CRS without nasal polyposis (CRSsNP) and CRS with nasal polyposis (CRSwNP) and allergic fungal rhinosinusitis (AFRS),[E1] its pathologic underpinnings are incompletely understood.[E2] *Refractory CRS* has been used to define cases that do not stabilize after surgery, antibiotics, saline rinses, and topical steroid treatment.[E3] *Recalcitrant CRS* has been used to define cases of recurrent nasal polyps after polyp surgery.[E4] This review will discuss the underlying infectious, innate, and adaptive immune components that characterize CRS, with special reference to "refractory" and "recalcitrant" cases.

Pathologic features of CRS

The pathologic appearance of CRS in adults is typically one of chronic inflammation with mixed mononuclear cells and variable numbers of eosinophils. Neutrophilic inflammation is more common in patients with CRSsNP, whereas eosinophilic inflammation is more typical of patients with CRSwNP, but there is overlap, with many patients with CRSsNP showing some degree of tissue eosinophilia.[E5-E7]

The microbiology of CRS

Role of viruses. There is limited information indicating that patients with CRS have an increased incidence of rhinovirus infection,[E8] but there is no evidence for persistent viral infection in the majority of cases.

Bacteriology of CRS by means of conventional culture techniques. Recent studies obtaining intraoperative sinus cultures with simultaneous analysis of cultures and biofilm in cases of refractory CRS reported positive cultures in 72.6% to 80% of cases, with a predominance of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in the isolates.[E9-E11] Atypical bacteria are rarely present.[E12]

Bacterial biofilm in patients with CRS. Biofilm formation is an important survival mechanism for microorganisms through attachment to surfaces.[E13] Biofilm represents a distinct ecological structure formed when bacteria produce a sugary macromolecular substance, which is referred to as extracellular polymeric substances or glycocalyx, that allows them to form a complex, 3-dimensional, resilient community attached to a surface. Bacteria within biofilm are in a "sessile" state, whereas free-floating bacteria are in the "motile" or "planktonic" state.* Biofilm on sinonasal mucosal surfaces was first described in 2004[E14] and later in several subsequent publications.[E15-E20]

Scanning electron microscopy and transmission electron microscopy provide ultrastructural confirmation of mucosal biofilm. Confocal scanning laser microscopy has the advantage that specimens can be imaged without fixation or dehydration, and specific bacteria or fungi can be identified with fluorescent markers. The fluorescent *in situ* hybridization assay uses either universal bacterial probes, such as EUB338,[E21] or species-specific primers based on unique sequences in the 16S ribosomal RNA gene. When studies with either scanning electron microscopy, transmission electron microscopy, or confocal scanning laser microscopy are taken in total, the prevalence of biofilm in patients with CRS is approximately 56%.

The presence of bacterial biofilm, particularly polymicrobial biofilm or biofilm containing *S aureus*, is associated with more severe preoperative sinus disease (worse radiologic and symptom scoring) and worse symptom and nasal endoscopy scores after sinus surgery.[E17,E18,E22,E23]

Abnormalities of the microbial community in the nose and paranasal sinuses. The microbial community can be defined as a multispecies assemblage of all culturable and nonculturable bacteria living together in a contiguous environment and interacting with each other.[E24] Studies of the microbial community in patients with CRS by using molecular techniques, such as pyrosequencing, have reported a higher abundance ("bacterial burden") of *S aureus* sequences in patients with CRS.[E25]

Role of fungal colonization and fungal biofilm in patients with CRS. Virtually all patients with CRS and healthy control subjects also have a positive fungal culture of nasal secretions, with a broad array of fungi isolated.[E26,E27] Studies with molecular techniques suggest that CRS is associated with an increased "fungal burden," most notably an increased burden of colonization with *Alternaria* species.[E28]

Barrier function in sinus epithelium and its relevance to CRS. Epithelial barrier function is important for maintaining mucosal hydration and preventing penetration of microbes into the subepithelial layer. Defective epithelial barrier function (loss-of-function mutations in the filaggrin gene) is an important risk factor for the development of atopic dermatitis; however, no defects in epithelial tight junction proteins have been described in patients with CRS.

Mucociliary clearance and its relevance to CRS. Mucociliary clearance is an essential process in normal sinus function. Active CRS is associated with a reduction in mucociliary clearance, but mucociliary clearance usually normalizes after clearance of infection and restoration of sinus ostial patency. There is no evidence for a primary defect in mucociliary clearance to account for CRS, except in cases of primary ciliary dyskinesia.

Host defects in innate immunity associated with CRS

Key antimicrobial proteins and peptides in host innate immunity. Nasal and sinus secretions contain a broad array of proteins and peptides with antimicrobial properties, including lysozyme, secretory leukocyte proteinase inhibitor, complement components C3 and serum amyloid A, ficolins, collectins, and the defensin and cathelicidin families of antimicrobial peptides. Decreased levels of lactoferrin were reported in sinus tissues of patients with refractory CRS,[E29,E30] whereas the levels of other antimicrobial proteins and peptides were reported to be normal.

Nitric oxide (NO) is believed to have antimicrobial effects in the upper airway. This might be due to stimulation of increased ciliary beat frequency but might also relate to complex reactivities between NO radical superoxide, metals, and thiols.[E31] High NO levels are constitutively produced in nasal and sinus epithelium (mean nasal NO level in healthy subjects, 233 ppb).[E32] No defects in NO production have been described in patients with CRS.

Innate signaling mechanisms through epithelial cells. Our understanding of the mucosal innate immune response

*Please refer to the Montana State University, Center for Biofilm Engineering Web site (www.erc.montana.edu/biofilmbook) for an excellent explanation and illustrations of biofilm.

in patients with CRS remains quite rudimentary, and more studies are needed to correlate innate immune function with microbial features, such as the presence of bacterial or fungal biofilm. The pattern recognition receptors involved in microbial recognition by sinus airway epithelial cells, their microbial ligands, and abnormalities described in patients with CRS are summarized in Table E1.[E4,E33-E41]

Microbial recognition through Toll-like receptors. Sinonasal epithelial cells express Toll-like receptors (TLRs) 1 through 10.[E42,E43] TLR2, TLR3, TLR4, and TLR9 signaling was demonstrated in sinonasal epithelial cells.[E33,E44] TLR ligation in airway epithelial cells results in activation of specific intracellular signaling pathways (as reviewed by Bals and Hiemstra[E34]), leading to (1) production of innate antimicrobial peptides and (2) production of cytokines and chemokines that amplify innate responses (eg, neutrophil infiltration) and activate adaptive immune responses. The TLR2 pathway has the greatest diversity of ligands and recognizes a wide array of gram-positive and gram-negative bacteria, as well as fungi, in part because of formation of heterodimers between TLR2 and TLR6. The TLR4 pathway is important in host responses to gram-negative bacteria.

Studies of TLR signaling pathways have focused on patients with refractory CRS and those with "recalcitrant" nasal polyposis. Increased expression of TLR2 was found in patients with recalcitrant CRS.[E35] Baseline expression of TLR9 was found to be reduced by 50% in cultured epithelial cells from patients with recalcitrant nasal polyposis,[E44] and the T_H2 cytokines IL-4 and IL-13 were shown to downregulate TLR9 expression in cultured normal sinus epithelial cells.[E4]

Microbial recognition through bitter taste receptors. Bitter taste receptors are a family of G protein–coupled receptors that signal by inducing a transient intracellular calcium flux. Activation of the receptor induces production of NO and increases ciliary beat frequency in sinus epithelial cells.[E36] A polymorphism (TAS2R38 variant) was discovered that is associated with reduced signaling, reduced NO production, reduced ciliary beat frequency, and increased growth of *P aeruginosa* in cultures of human airway epithelial cells,[E36] suggesting a mechanistic link between deficient innate signaling and increased bacterial infection.

Adaptive immune antimicrobial signaling mechanisms: IL-17A and IL-22 signaling pathways

IL-22 is an "essential guardian of mucosal immunity against extracellular bacteria in the lung and gut."[E45] It stimulates the production of a wide variety of antibacterial proteins and mucin 1 production under inflammatory conditions and enhances epithelial regeneration with goblet cell restitution.[E46] $CD4^+$ T_H17 cells produce IL-22 in conjunction with IL-17A and IL-17F.[E47] A subset of $CD4^+$ T_H cells that produce IL-22 but not IL-17 has also been identified in human subjects (T_H22 cells).[E48] No specific defects in IL-17A or IL-22 signaling have been identified in patients with CRS.

Role of bacteria or fungi in maladaptive T_H2 responses in patients with CRS

CRS is a disease in which the local tissue inflammatory response is strongly biased toward T_H2 inflammation. This is particularly true in patients with CRSwNP[E49] but also true to a lesser degree in patients with CRSsNP.[E5-E7,E50] There is evidence linking colonizing microorganisms to this maladaptive T_H2 "local allergic" response.

Fungi, particularly *Alternaria* species, are commonly detected in the attached mucus of sinus tissues in patients with CRS[E28,E51] and can induce eosinophil activation and degranulation.[E52] *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. Fungal allergens also elicit modest production of IL-5 and IL-13 from dispersed nasal polyp T lymphocytes.[E53] Thus colonizing fungi might contribute to maladaptive T_H2 mucosal immune responses.[E51]

Mucosal colonization with *S aureus* has been found in 64% of patients with CRSwNP compared with 30% of healthy subjects or patients with CRSsNP.[E54] In a study of 13 patients with massive polyposis, 55% of patients were found to have enterotoxin-producing *S aureus* in the mucus adjacent to polyps.[E55] Furthermore, T lymphocytes isolated from polyps show a skewing of Vβ usage, with enrichment for Vβ known to respond to staphylococcal superantigens.[E55-E57] IgE antibodies directed against staphylococcal superantigens are present in nasal polyp homogenates in 27.8% of patients with nasal polyps and 53.8% of patients with nasal polyps with coexisting asthma.[E54] Finally, staphylococcal enterotoxin B induces robust production of IL-5 and IL-13 in dispersed nasal polyp T lymphocytes.[E53] These studies suggest that colonizing *S aureus* might be a major driver of the local T_H2 inflammatory response in patients with CRSwNP.

Downregulation of epithelial innate immunity by maladaptive T_H2 tissue inflammation

A downregulation of epithelial innate immunity by maladaptive T_H2 tissue inflammation has been demonstrated in patients with recalcitrant CRSwNP. *In vitro* studies have shown that culture of normal primary nasal epithelial cells with IL-4 and IL-13 decreased TLR9 expression by nearly 50% and also reduced expression of human β-defensin 2 and surfactant protein A.[E4] Colonizing fungi might contribute to this downregulation by inducing maladaptive T_H2 responses.

THE CASE REVISITED

This patient has "recalcitrant" nasal polyposis, allergic mucin, and evidence of a strong maladaptive T_H2 eosinophilic inflammatory response. Allergic mucin is a hallmark feature of AFRS, but it can also be seen in patients with CRSsNP and CRSwNP.[E58] The pathologic description of "polypoid chronic sinusitis with stromal eosinophilia" indicates the presence of an intense maladaptive T_H2 response. This patient also had a positive bacterial culture for "few" *S aureus* and *P aeruginosa*. Their presence might contribute to the T_H2 maladaptive response or be partially the result of decreased innate immunity in the face of marked T_H2 inflammation, as discussed previously.

Given the intensity of eosinophilic inflammation and the "few" bacteria in this case, the significance of the bacteria in the disease process could be questioned. The tissue was not specifically studied for biofilm. *S aureus* has been shown to persist in the sinuses after endoscopic sinus surgery, even in culture-negative cases in which *S aureus* biofilm was detected at the time of surgery.[E59] Given this and the fact that colonizing *S aureus* plays a key role in maladaptive T_H2 responses in patients with nasal polyps, *S aureus* can certainly play an important role in cases of recalcitrant CRS.

This patient also has 2 of the 3 major criteria for AFRS, namely the presence of allergic mucin and positive allergy test results to fungus. The result of the IgE RAST test for *A fumigatus* was also

strongly positive. What is lacking for AFRS is a positive fungal stain or culture result. Fungi can be difficult to identify in mucus specimens from sinus surgery because of the insensitivity of the Grocott methenamine silver stain and technical difficulties with the culture.[E60] Patients such as this have been described as having "eosinophilic mucin rhinosinusitis"[E61] or being "AFRS candidates."[E62] Treatment with systemic steroids is useful in such cases and similar in either eosinophilic mucin rhinosinusitis or "candidate AFRS" cases. Topical steroid irrigations are also especially helpful after sinus surgery.[E58] In contrast, neither topical nor systemic antifungal agents have been shown to be effective.[E63] In fairness, the clinical trials of these medications have never specifically studied patients with eosinophilic mucin rhinosinusitis or "candidate AFRS" patients. This highlights the need for more precise characterization of CRS subsets based on host-microbial features, such as the presence of biofilm, the presence of fungi, underlying defects in innate immunity, and the nature of the underlying maladaptive T_H2 response. More precise characterization is likely to happen in the future as these factors are used as entry criteria for clinical trials, and the result should mean a more targeted and effective approach to therapy.

In this case the patient was advised to initiate a sinus rinse with budesonide concentrated solution (0.5 mg in 7 mL of saline per nostril) daily in the head-down forward, lateral supine, and supine positions.[E58] She was also advised to install dust mite–proof encasings. The plan is to see her again in 3 months to assess rhinoscopically how well the rinses are working and whether she has any persistent evidence of infection.

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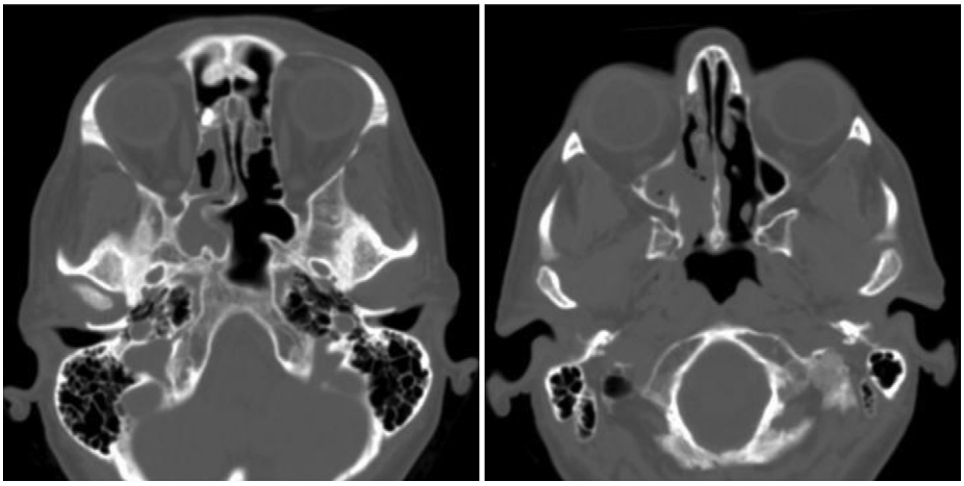

FIG E1. Representative axial sections of a head computed tomographic scan taken preoperatively that revealed no intracranial abnormalities but extensive mucosal thickening in the right maxillary sinus, right ethmoid air cells, and right sphenoid. Coronal views were unavailable.

TABLE E1. Pattern recognition receptors involved in microbial recognition by airway epithelial cells, their microbial ligands, and abnormalities described in patients with CRS*†

Receptor	Ligand	Relevant pathogens	Key innate signaling events in airway epithelial cells	Abnormalities described in patients with CRS
TLR2	Peptidoglycan, lipoteichoic acid, and lipoprotein from gram-positive bacteria, lipoarabinomannan from mycobacteria, and zymosan from yeast cell wall	Gram-positive and gram-negative bacteria; fungi (*Candida* species and *Aspergillus fumigatus*)	Increased production of hBD-2 and IL-8	Increased expression of TLR2 in patients with recalcitrant CRS[E35]
TLR3	Viral double-stranded RNA (polyinosine-polycytidylic acid is a synthetic analog of double-stranded RNA)	Rhinovirus, other viruses	Increased type I and type III interferon levels; chemokines (IL-8, GRO-α, RANTES, CXCL10); hBD-2 and hBD-3	Exaggerated response to TLR3 plus cigarette smoke extract with excess production of RANTES and hBD-2[E33]
TLR4 (including CD14 and MD2 on cell surface)	LPS (facilitated by LPS-binding protein)[E37]	Gram-negative bacteria; *Candida* species and *Aspergillus fumigatus*[E38]	Nuclear factor κB and activation of proinflammatory cytokine genes, including IL-8 and hBD-2	Reduced expression of TLR4, TLR7, and MyD88 in patients with CRSsNP compared with control subjects[E39]
TLR7/TLR8	Single-stranded RNAs (natural ligands); small synthetic molecules: imidazoquinolines and nucleoside analogs	Viruses	Nuclear factor κB and activation of proinflammatory cytokine genes	Reduced expression of TLR4, TLR7, and MyD88 in patients with CRSsNP compared with control subjects[E39]
TLR9	Specific unmethylated CpG oligonucleotide sequences (CpG DNA)	Bacteria	Production of IL-8[E40]	Decreased baseline expression of TLR9 in patients with CRSwNP; decreased TLR9 expression in cultured AECs in response to IL-4 and IL-13[E4]
Bitter taste receptors	Functional responses to pathogen-derived quorum-sensing molecules	*Pseudomonas aeruginosa*	NO production, stimulation of mucociliary clearance, and direct antibacterial effects	TAS2R38 genotype correlated with *Pseudomonas aeruginosa* infection in patients with CRS[E36]

AEC, Airway epithelial cells; *CpG*, bacterial DNA containing unmethylated CpG dinucleotides; *GRO-α*, growth-regulated oncogene α; *hBD*, human β-defensin; *LPS*, lipopolysaccharide; *MyD88*, myeloid differentiation primary response gene–88; *PAMP*, pattern-associated molecular motif.
*Adapted from Bals and Hiemstra,[E34] Ooi et al,[E41] and Roeder et al.[E38] TLR ligand information source: http://www.invivogen.com/tlr2-ligands.
†TLR1, TLR5, TLR6, and TLR10 are not included because there are no reports of abnormalities in these TLRs associated with CRS.