

Bacterial biofilms and chronic rhinosinusitis

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ABSTRACT

Chronic rhinosinusitis (CRS) is a highly prevalent disease in the adult and pediatric population. It causes significant burden and the management is considered one of the most costly public health conditions. Comorbidities include asthma, aspirin sensitivity, and nasal polyposis. Staphylococcus aureus biofilms and exotoxins that act as superantigens have been implicated to play an important pathological role in the incidence, maintenance, and ongoing burden of CRS. A better understanding of the interplay between bacterial factors, host factors, and the environment will facilitate better management of this disease. This literature review focuses on these factors and highlights current research in this field.

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It is estimated that 37 million adults in the United States suffer from chronic rhinosinusitis (CRS).¹ It is highly prevalent across all racial groups.² Approximately \$3–5 billion is spent on this disease annually.³ An estimate in 1999 placed CRS as part of the top 10 most costly physical health conditions.⁴ The total aggregate workdays missed in the United States is estimated at 11.5 million.⁵ CRS is notoriously difficult to control. Comorbidities include asthma, aspirin sensitivity, and nasal polyposis. Serious complications include orbital cellulitis, meningitis, and brain abscess.⁶

BACTERIAL BIOFILMS

Bacteria can be found in two distinct phenotypes: planktonic, which consists of free-floating single cells, or biofilms, which are a dormant adaptive mode that supports the viability of a bacterial community.⁷ Biofilms are a three-dimensional communicating organization of bacterial cells surrounded by a protective extracellular water-based glycocalyx matrix. Pores within this organized structure are conduits for nutrient and waste exchange. Bacterial cells within these biofilms are more resistant to host defenses such as immune system phagocytosis and can be up to 1000 times more resistant to antibiotics.^{8,9} Biofilm growth is associated with an increased level of mutations. Antibiotic resistance is likely enhanced by the ability of resident bacteria cells to share genetic material within the biofilm structure, thus facilitating the persistence of antibiotic-resistant genes.^{10,11} Diseases associated with biofilms are characterized by persistent infection, with

intermittent periods of acute exacerbations.¹² Infection is supported as viable bacteria detach from the films. Cells in the biofilm are in a dormant state, allowing them to remain viable with minimal nutritional support. There is growing evidence that bacterial biofilms may play a role in certain cases of recalcitrant CRS that do not respond to traditional medical and surgical therapies.^{13,14} Bacterial biofilms have been implicated in the ongoing burden of CRS.¹⁵ They have an adverse effect on postoperative outcomes in CRS patients and predict poor prognosis.^{13,16}

The mechanism of biofilm formation was studied in cystic fibrosis patients with *Pseudomonas aeruginosa*.¹⁷ Cell-to-cell signaling controls expression of virulence factors. Quorum sensing (QS) is a cell-to-cell signaling mechanism that refers to the ability of bacteria to respond to hormone-like molecules called autoinducers. Water channels within the biofilm mediate interbacterial QS. The role played by the QS systems was investigated during the early stages of static biofilm formation when cells are adhering to a surface. Genes for the QS systems were maximally expressed in these cells and expression decreased with increasing biofilm height.

Bacteria that cause device-related infections grow in matrix-enclosed biofilms.¹⁸ To address this, Lellouche described a water-based synthesis of nanoparticles using sonochemistry.¹⁹ Nanoparticle-coated catheters were able to reduce bacterial colonization, highlighting their potential role as antibiofilm agents.¹⁹ This may be caused by an early evoked immune response. This has been observed with the effects of nanomaterials on tissues. A review of the literature revealed an inflammatory response with increased reactive oxygen species to be common immune responses to nanomaterial used for drug delivery systems.²⁰

S. AUREUS AND BIOFILMS

Nasal *Staphylococcus aureus* carriage has been linked to community-acquired and nosocomial infections.²¹ Bac-

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terial resistance mechanisms, together with host immune systems, contribute to nasal colonization as the bacteria adapts to the host environment.²² Both host genetics, such as mucociliary dysfunction, and nasal anatomy, such as nasal polyps, and deformation of the middle meatus, as well microbial genetics play a role. Bacterial strains from the carrier host develop a competitive advantage as it adapts to evade host defenses allowing colonization. This leads to increasingly more resistant bacteria with reduced antibiotic sensitivity. Studies have shown that *S. aureus* biofilms and polyps are associated with specific changes in host gene expression, further establishing this complex interplay.²³ The *S. aureus* isolated from nasal carriers are associated with an elevated neutrophil-mediated inflammatory response.²⁴ Biofilm and planktonic cultures of *S. aureus* have different impacts on gene expression in keratinocytes, implying that biofilms can influence host gene expression.²⁵

The carrier strain of *S. aureus* is associated with biofilm formation.^{24,26} Moreover, the carrier strain biofilm exoproteome contained a greater number of immunoevasive proteins than its planktonic counterpart.²⁷ This likely contributes to the proper environment to allow the planktonic phase to thrive. Recent studies on staphylococcal living conditions during nasal colonization favor a dispersed rather than a biofilm-related mode of growth during *S. aureus* nasal colonization.²⁸ Thus, it seems that biofilms act as a bacterial resting state that can differentiate into the planktonic free-living phenotype that drives chronic infection. Conditions or triggers that induce this detachment are currently unknown.

SINUSITIS PHYSIOLOGY

CRS represents a persistent inflammatory state that includes activation of eosinophils and constant up-regulation of the proinflammatory cytokines.²⁹ This inflammation is supported by the interplay of host and bacterial factors. Host factors include anatomic such as ostial obstruction and polyposis, genetic such as innate host defenses, and the general health condition. Phenotypical classification considers the presence of nasal polyps and mucosal eosinophils.³⁰ Genetic deficiencies in mannose-binding lectin and IgA can lead to severe infection requiring multiple antibiotics that are prevented from reaching the sinuses by biofilm formation.³¹

Patients with a predisposition for allergies are more likely to suffer from CRS. The binding of allergens to IgE on mast cells causes release of cytokines and inflammatory mediators in nasal secretions. Cytokine levels tend to peak 3–4 hours after an antigen challenge.³² The subsequent chronic inflammatory response can lead to genetic alterations leading to per-

manent tissue damage and symptoms that become more difficult to control.³³ CRS is associated with changes at the genetic level that contribute to the chronic inflammatory reaction.³⁴ Thus, the goal is targeted early treatment during the initial inflammatory response before permanent changes at the genetic level occur. Biofilms can be prevented by early aggressive antibiotic therapy.¹¹

CRS with nasal polyps tends to be refractory to treatment. These patients were more likely to have exotoxin-producing *S. aureus* adjacent to polyps.³⁵ These exotoxins may act as superantigens causing clonal expansion of nonspecific T lymphocytes with massive cytokine production. The lateral wall of the nose in patients with polyposis appears to be an ideal environment for the development of this superantigen reaction.³⁶ Staphylococcal superantigens can modify the immune response, contributing to persistent severe disease.³⁷ This drives IgE production and polyclonal eosinophilic activation worsening inflammation. The superantigen hypothesis is more highly accepted than chronic infection driving the pathogenesis of CRS.³⁸ CRS driven by chronic infection is usually associated with an underlying immunodeficiency.³⁹

ROLE OF NITRIC OXIDE

Nitric oxide (NO) is a ubiquitous physiological messenger. Important functions include vasodilatation, smooth muscle relaxation, regulation of inflammation, and innate immunity. It is known to have a dual role in inflammation, because it can both positively and negatively regulate the levels of cytokines. Thus, it is associated with both protective and damaging effects.⁴⁰ The molecular details are still being investigated.⁴¹ The paranasal sinuses are a well-established reservoir of NO in healthy adults.⁴² NO is generated by epithelial cells in both the upper and the lower airways. NO liberation can be triggered by exacerbations of allergic rhinitis and sinusitis.^{43,44} Interleukin (IL)-8 and NO levels have been detected in the nasal discharge of patients with sinusitis.^{45,46} H₁-antagonists or nasal steroids cause reductions in nasal IL-8, neutrophils, and eosinophils.^{47,48} Chemotaxis of eosinophils is triggered by the increase in nasal NO contributing to the oxidative stress in reactive airways.⁴⁹ IL-33 has also been recently implicated in the allergic inflammatory response.⁵⁰ This has provoked an interest in targeting cell signaling pathways for allergic rhinitis therapy. NO is involved in ciliary motion and antibacterial defense and nasal levels may be normal, raised, or lowered in disease states. Low levels are associated with ciliary dyskinesia, which can lead to severe chronic sinusitis and bronchiectasis.⁵¹ It has been observed that exhaled NO is decreased in cystic fibrosis, nasal polyposis, acute rhinosinusitis, and CRS. Alterations in the ex-

pression of genes involved in NO regulation have been associated with *S. aureus* biofilms and nasal polyps.⁵² Further investigations are needed to understand the clinical relevance of this observation.

An increased concentration of NO has been reported in bronchial asthma and nasal allergy, where its concentration is related to bronchial hyperresponsiveness and sputum eosinophilia.⁵³ Highest values are associated with acute asthma exacerbations. Corticosteroid administration is effective in reversing the induction of NO synthesis.⁵⁴ These observations highlight the diverse role of NO, indicating the importance of correlating NO levels with clinical disease. Acute hyperactive airway reactions appear to correlate with elevated NO levels whereas diffuse chronic conditions are more associated with low NO levels. Thus, measuring NO levels can help narrow the differential diagnosis and guide management of airway diseases.

NO levels are increased after successful treatment of CRS; thus, it is a potential biomarker for inflammation to monitor treatment.^{55,56} It has been used as a biomarker for allergic rhinitis and asthma.^{44,57-59} Levels are modulated by nasal glucocorticoids.⁶⁰ Collection of nasal secretions using a filter paper matrix provides a reproducible model for detecting changes in cytokine levels after nasal challenge.³² A noninvasive method to measure exhaled NO concentration has been developed that enables physicians to assess airway inflammation in the office.^{40,53} Measuring both bronchial and nasal NO may assist the management of upper and lower airways.⁵³ These tests are also being investigated for the use in chronic obstructive pulmonary disease management.⁶¹

It has been observed that principal cytokines, such as IL-4, IL-8, TNF- α , and interferon γ , may be helpful for monitoring treatment and prognosis for patients with allergic rhinitis.⁶² These measurements may also be useful for patients with CRS, nasal polyps, and cystic fibrosis, as well as in the diagnosis of primary ciliary dyskinesia. NO also plays an important role in the bronchial inflammatory response in cystic fibrosis. *P. aeruginosa* lipopolysaccharide, and *Aspergillus fumigatus*. Recombinant, human deoxyribonuclease, a therapeutic agent that decreases sputum viscosity, has been shown to decrease nitrite in these stimulated cells.⁶³ Clinicians must always be aware whether or not they are treating a local or diffuse inflammatory response. A localized mucosal allergy will respond differently to treatment compared with allergic rhinitis, which is more of a diffuse chronic condition.⁶⁴

Bacteria that colonize the nose are a source of NO.^{65,66} The relationship between sinonasal NO and *S. aureus* colonization is still an active area of research. High NO levels have been measured in healthy sinuses and likely contribute to maintenance of relative sterility.⁶⁷ An antibiofilm effect is also observed at higher

concentrations.¹⁵ The lower concentrations found in the sinuses of CRS patients may decrease this effect and thus facilitate the biofilm formation. The observations that NO increase in sinonasal tracts during an inflammatory response in CRS and at the same time may act as a defense against chronic infection in healthy sinuses underscores the dual role played by this diverse molecule. In addition, polymorphisms in the NOS1 gene may play a role in the susceptibility and severity of CRS.⁶⁸

CLINICAL

Acute rhinosinusitis is usually viral with symptoms that self-resolve in <4 weeks. Subacute symptoms last between 4 and 8 weeks.⁶⁹ CRS is characterized by symptoms of nasal irritation, rhinorrhea, and nasal blockage with the concomitant presence of pressure or pain lasting >12 weeks or occurring more than four times per year.⁷⁰ Imaging is important to correlate symptoms with sinus pathology. A combination of clinical signs and symptoms, nasal endoscopy, and CT is usually needed to make the diagnosis of chronic sinusitis.⁷¹ Nasal endoscopy is a diagnostic tool commonly used for adenoiditis that is also helpful for detecting bacterial biofilms.⁷² This tool is particularly useful in children and postsurgical patients.^{73,74} Two reported cases, by the author (M.F.), reveal examples where biofilm formation may have played a role. The first case was an uncommon immune deficiency in a patient with frequent sinus infections for 15 years and the second case was a patient with chronic sinusitis and steroid resistant cough-induced asthma. Both patients required multiple courses of antibiotics, which could have been related to biofilm formation.^{75,76}

It has been known for a long time that macrolide therapy has a good clinical response for acute bacterial sinusitis.^{73,74} Currently, macrolides are the drug of choice for acute sinusitis.^{75,76} Erythromycin has been shown to inhibit the motile ability of neutrophils, suggesting that macrolide antibiotics may inhibit the positive feedback of IL-8 and neutrophil recruitment.⁷⁷ The main criterion for the efficacy of this treatment was variations of IL-4, IL-8, TNF- α , and interferon γ levels. Thus, macrolides appear to affect the inflammatory process and this may be the reason why they can be helpful in CRS. In 17 patients with CRS that persisted after sinus surgery, long-term, low-dose erythromycin therapy was found to be a promising approach based on clinical outcome and effects on nasal NO, ciliary beat frequency, and mucociliary transport.⁷⁸ This study suggested that long-term, low-dose treatment with erythromycin may be effective in persistent disease that does not respond to surgery or other medical therapies. On the other hand, antifungal treatment has not been so promising.⁷⁸

Clarithromycin therapy is known to alter bacterial-specific cytokines and NO levels in acute sinusitis. The efficacy of macrolide therapy was reduced in patients with polyposis. Nasal polyps are associated with eosinophilia; thus, these patients particularly benefit from the early eosinophil targeting provided by macrolides.⁷⁹ Polypectomy resulted in significant improvement in the efficacy of therapy. Nasal steroids also help with the associated edema.⁸⁰ CT scan findings were also helpful for identifying disease that is less responsive to macrolides.⁸¹ Antimicrobial photodynamic therapy is a nonantibiotic broad-spectrum treatment that has been shown to eradicate antibiotic-resistant bacteria and biofilms.⁸² Human clinical studies are currently planned to assess the safety and efficacy. Long-term antibiotics provide selective pressure for resistant bacteria. Topical and inhaled administrations are used to reduce this occurrence. Inhaled thiamphenicol associated with acetylcysteine has shown some promising results for bacterial rhinopharyngitis in adults and children.^{83,84} Low-frequency ultrasound has shown some promise in the treatment of bacterial biofilms associated with CRS.⁸⁵ This method delivers topical solutions to the nasal epithelium. Endoscopic sinus surgery is another option for CRS patients. Although the procedure is relatively safe, patients with nasal polyps and asthma are at risk for perioperative complications such as cerebral spinal fluid leakage.^{86,87} In particular *S. aureus* biofilms may predict a poorer response to surgery.⁸⁸ Maxillary sinus antrostomy tubes that selectively irrigate paranasal sinuses with topical antibiotics and anti-inflammatory agents is an option for recalcitrant CRS.⁸⁹

FUTURE PROSPECTS

CRS is a highly prevalent disease that involves the interplay between host, genetics, and infection. There is considerable evidence that biofilms play a role in the pathogenesis.^{14,87} More work is still needed to understand the association of biofilms and recalcitrant CRS. Current research is focusing on a better understanding of the interplay between bacterial superantigens, biofilm adherence, host defense, and anatomic features such as polyposis.⁹⁰ The molecular details of the inflammatory response are also still being investigated, because it is important to understand the immune response to the presence of biofilms.⁹¹ This can lead to better diagnostic markers and therapeutic interventions targeting inflammatory signaling pathways.⁹² Studies to understand how the presence of biofilms can help guide management and aid in prognosis are also needed.^{93,94} Clinical risk factors such as asthma, polyposis, nasal steroids, sinus surgery, and atopy also need further investigation. In particular, recent studies have identified a correlation between asthma severity

and CRS progression.^{95,96} Animal models of CRS have been constructed with some promising results but still more data are needed.⁹⁷⁻¹⁰⁰ In addition, studies to clarify the role of NO measurements in airway diseases are still active. Further research is aimed at investigating its role in allergic rhinitis and nasal congestion.⁵³ Several questions still need to be addressed, in particular the evaluation of the sensitivity and specificity of the measurement technique and assessment of the bronchodilator action of endogenous NO.¹⁰¹

Nasal endoscopy is an important emerging tool for the diagnosis of CRS.¹⁰² Additional investigations are needed to standardize its interpretation to improve the quality of this procedure.¹⁰³ Noninvasive detection of biofilms is another area of current investigation. An immunofluorescence protocol to detect the biofilm matrix polysaccharide is currently being developed.¹⁰⁴ Long-term antibiotics have led to resistance and unsatisfactory results, prompting investigations into other treatments.⁷¹ Drugs that interfere with biofilm formation are being investigated, particularly to target the cell-to-cell QS system.^{90,105,106} This system is thought to be important for *S. aureus* superantigen formation. Drugs that target bacterial adherence are also being developed.⁹⁰ Topical mupirocin was found to be effective in reducing biofilm mass.¹⁰⁷ These studies indicate that the focus for treatment should be biofilm disruption rather than bacterial killing. Eradicating the bacteria without the biofilm leaves the potential for future infections. Another generation of bacterial cells can inhabit the biofilm, allowing infection to persist, likely even more virulent because time allows for more resistant mutations.

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