Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Curr Otorhinolaryngol Rep. Author manuscript; available in PMC 2019 September 01.

Published in final edited form as:

Curr Otorhinolaryngol Rep. 2018 September ; 6(3): 253–262. doi:10.1007/s40136-018-0212-6.

Understanding the Role of Biofilms and Superantigens in Chronic Rhinosinusitis

Ivy W Maina, BA[#1,2], **Neil N Patel, BA, BS**[#1,2], and **Noam A Cohen, MD, PhD**[1,2,3,*]

[1]Department of Otorhinolaryngology-Head and Neck Surgery, Division of Rhinology, University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA.

[2]Philadelphia Veterans Affairs Medical Center, Philadelphia, PA.

[3]Monell Chemical Senses Center, Philadelphia, PA.

[#] These authors contributed equally to this work.

Abstract

Purpose of review: This review explores recent discoveries in our understanding of how biofilms and superantigens contribute to the pathogenesis of chronic rhinosinusitis (CRS). It also examines clinical implications and novel treatment approaches for biofilm associated CRS.

Recent findings: While the role of biofilms in CRS has been studied for 14 years, research interest has now turned toward elucidating new methods of biofilm detection, microbial diversity, and novel treatment approaches. Recent studies on biofilm superantigens aim to clarify the immunological mechanisms of upper airway inflammation, particularly the type-2 response seen in nasal polyposis.

Summary: Biofilms are a topic of research interest for their role in the pathogenesis of chronic rhinosinusitis, particularly when they elute superantigens. New studies on this topic focus on the molecular and cellular mechanisms at play.

Keywords

biofilm; superantigen; chronic rhinosinusitis; innate immunity; staphylococcus aureus; nasal polyposis

[*]Corresponding Author: Noam A. Cohen M.D. Ph.D., Division of Rhinology, Department of, Otorhinolaryngology-Head and Neck Surgery, University of Pennsylvania Medical Center, 5th Floor Ravdin Building, 3400 Spruce Street, Philadelphia, PA, 19104, Tel: 215-823-5800 ext 3892, Noam.Cohen@uphs.upenn.edu.

Compliance with Ethics Guidelines

Disclosure of potential conflict of interest: IWM and NNP have no relevant conflicts to this manuscript to disclose. NAC has a patent pending “Therapy and Diagnostics for Respiratory Infection.”

Human and Animal Rights and Informed Consent

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the studies involving human subjects.

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Introduction

Chronic rhinosinusitis (CRS) is a complex and common syndrome affecting up to 16% of the United States population [1]. CRS invokes a direct treatment cost estimated between $10–13 billion per year in the US and CRS patients suffer from significantly impaired quality of life [2]. Though a broad syndrome, CRS is often classified into two phenootypes based on the presence or absence of nasal polyps on nasal endoscopy. CRS without nasal polyps (CRSsNP) is characterized by a T helper type 1 (Th1) mediated pattern of inflammation and CRS with nasal polyps (CRSwNP) has greater propensity for a T helper type 2 (Th2) mediated immune response [3].

Despite its impact on a societal and individual level, the aetiology of CRS remains unclear. In recent years, research has focused on the multifactorial interactions of host and environmental factors that determine susceptibility to CRS. Examples of host factors include genetic disorders affecting mucociliary clearance like cystic fibrosis and primary ciliary dyskinesia, while environmental factors include pathogens, allergens and tobacco exposure. However, the majority of cases of CRS are idiopathic and multiple hypotheses have been proposed to explain the pathophysiology of CRS, including the potential role of biofilms and superantigens. The purpose of this review is to explore recent advances in our understanding of the biofilm and superantigen hypotheses, examine the clinical implications of biofilms and superantigens associated CRS, summarize emerging interventions based on these hypotheses and suggest future directions for research.

The Biofilm Hypothesis

Biofilm Formation

Bacteria can exist in two distinct forms— planktonic or biofilm— which demonstrate differential growth and gene expression patterns. In planktonic form, bacteria are free floating; in biofilm form, the state in which 99% of bacteria prefer to exist, bacteria form closely organized microcolonies encased in extracellular matrix (ECM) [4]. Biofilm formation occurs when planktonic bacteria irreversibly adhere to a surface via the upregulation of cell adhesion structures and proliferate to form microcolonies which secrete a protective layer formed from polysaccharides, proteins, nucleic acids, and extracellular DNA [4]. Once a critical mass is reached, a phenomenon known as quorum sensing begins which allows bacteria within the biofilm to communicate and coordinate via small signal molecules called autoinducers. This bacterial cross-talk culminates in signal transduction cascades that alter gene expression leading to the final biofilm phenotype. Existing in biofilm form enhances bacterial survival via several mechanisms. First, the protective layer of ECM allows bacteria to evade detection by the host immune system [5]. Second, the exchange of extrachromosomal DNA is more efficient in the biofilm state, enabling the propagation of adaptive mutations [5]. Third, the ECM protection and the coordinated bacterial stress response via quorum sensing confer antibiotic resistance in biofilm form that is up to 1000 fold greater than in planktonic form [6]. Biofilms can then spread by releasing planktonic bacteria to distant sites which can adhere and begin the complex process of biofilm formation again [7].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Biofilm Detection

Biofilms have been implicated in multiple infectious processes significant to the practice of otolaryngology including otitis media [8] and chronic tonsillitis [9]. Recently, research has focused on investigating the prevalence and potential pathophysiological role of biofilms in CRS. Biofilms were first identified in CRS patients in 2004 [10] and several studies have subsequently reported biofilm prevalence ranging from 25% to 100% using a variety of detection methods [11] The earliest studies to attempt biofilm detection in CRS utilized nasal swab cultures which rely on selective growth methods that favor more abundant and metabolically active organisms. Given that bacteria in biofilm have reduced metabolic activity, traditional culture methods often sample planktonic bacteria, misrepresenting the apparent diversity of the microbial community in CRS [8]. Newer and more reliable modalities for biofilm detection include scanning electron microscopy (SEM), transmission electron microscopy (TEM), fluorescence in situ hybridization (FISH), and confocal scanning laser microscopy (CSLM), which is currently considered the gold standard.

Microbial Diversity

Since the advent of biofilm detection in CRS, multiple bacterial organisms have been implicated including *Staphylococus aureus, Pseudomonas aeruginosa, Haemophilus influenza* and *Moraxella cattarhalis* [8]. Of these, *S. aureus* biofilms have the greatest association with severely recurrent and recalcitrant cases of CRS [12]. Recently, the ability of *S. aureus* to produce superantigens, which induce a local and at times wide-spread non-specific immune response, has been thought to explain the enhanced pathogenicity of *S. aureus* compared to other bacterial species.

While research has mostly focused on the bacterial components of biofilms, several studies have established a high correlation between bacterial and fungal biofilms in patients with CRS, suggesting a potentially mutualistic relationship. In a sheep model of CRS, co-inoculation of the frontal sinus with *Aspergillus fumigatus* and either *S. aureus, P. aeruginosa* or *S. epidermidis* promoted fungal biofilm formation, while inoculation with *A. fumigatus* alone did not [13]. Co-inoculation with bacterial and fungal elements also led to greater degrees of inflammation and ciliary damage than fungal inoculation alone. This synergistic effect of bacterial and fungal organisms is thought to occur at least partially due to bacterial induced mucociliary injury which facilitates opportunistic infections by fungal species [13, 14].

Potential Mechanisms of Pathophysiology

Despite a growing body of evidence implicating biofilms in the pathogenesis of CRS, not all CRS patients demonstrate evidence of biofilm formation during their disease process. To further understand the potential role of biofilms in CRS it is necessary to explore the factors that dictate susceptibility to biofilm formation. Evidence suggests that host defects in innate and adaptive immunity drive susceptibility to biofilms leading to a subgroup of CRS patients with recurrent and recalcitrant disease.

The recent discovery of taste receptors in the upper airway has yielded evidence suggesting a potential role in protecting against biofilm formation. Bitter and sweet taste receptors are

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

G- protein coupled receptors which when stimulated activate the canonical taste signaling cascade [15]. Taste receptors belonging to Taste Receptor Family 1 subtypes 2 and 3 (T1R2/T1R3) detect sweet compounds while those belonging to Taste Receptor Family 2 (T2R) respond to a variety of bitter compounds [16]. In the upper airway, taste receptors are present on a variety of cell types and mediate components of innate immune defense. Ciliated cells express T2Rs and respond to bitter compounds including specific autoinducers involved in quorum sensing called acyl-homoserine lactones released by biofilm forming gram-negative bacteria such as *P. aeruginosa.* Stimulation of T2R38 results in a signaling cascade with resultant calcium- dependent release of bactericidal nitric oxide and increased ciliary beat frequency [17–19]. Similarly, solitary chemosensory cells (SCC) are rare epithelial cells that express both T1R2/3 and T2Rs. SCC T2R stimulation elicits a calcium response that results in the downstream release of antimicrobial peptides, while stimulation of T1R2/3 acts to antagonize this T2R response. During bacterial infection, glucose levels in mucus decrease thereby reducing stimulation of TIRs which disinhibits the T2R response to bacterial products [15].

Dysfunction of either bitter or sweet taste receptors in the upper airway can impair the detection of and response to quorum-sensing AHLs as evidenced by recent work investigating T2R38, one of the most well studied T2Rs. T2R38 is encoded by the *TAS2R38* gene and is particularly sensitive to the bitter compounds propylthiouracil (PROP) and phenylthiocarbamide (PTC) [20]. Genetic polymorphisms dictated by missense mutations in SNPs determine T2R38 functionality and, therefore, sensitivity to PTC and PROP. The T2R38 protein exists in two common haplotypes based on the amino acids encoded by missense mutations in single nucleotide polymorphism. One form, PAV (proline, alanine, valine) is functional and the other, AVI (alanine, valine, isoleucine) is non-functional. These two haplotypes result in three common genotypes and the level of PAV expression correlates with T2R38 receptor sensitivity to bitter compounds [21]. Sensitivity to PTC, as measured by a simple taste test, can serve as a proxy for *TAS2R38* genotype and T2R38 functionality. A recent study by Adappa et al. sought to investigate whether T2R38 function is implicated in biofilm formation given its role in detecting quorum sensing molecules. Endoscopic nasal swabs were obtained from CRS patients who were also evaluated for T2R38 functionality with a PTC taste test. An inverse linear relationship was found between *in vitro* biofilm formation and PTC sensitivity in the entire cohort, but this association was driven specifically by CRSsNP patients. These results suggest that differential T2R38 receptor activity determines the innate immune response in the sinonasal tract. Furthermore, taste tests using appropriate bitter compounds, like quinine hydrochloride and denatonium benzoate, at optimized concentrations have been shown to serve as a cost-effective and accurate proxy for sinonasal immune function [22].

Another component of the innate immune system that demonstrates dysfunction in biofilm associated CRS is the mucociliary apparatus. Through coordinated movement, ciliated cells eliminate mucus produced by goblet cells which traps pathogens traveling through the upper airway. In a rabbit model of sinusitis, Jia et al. traced the natural history of biofilm formation after bacterial inoculation with *S. aureus.* The earliest changes seen 1 week post-operatively were goblet cell hyperplasia with resultant increase in mucous production, followed by ciliary warping and concomitant reduction in ciliary activity [23]. Other studies have

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

demonstrated varying degrees of epithelial destruction ranging from ciliary disarray to complete absence of cilia [24, 25]. It is unclear whether this ciliary dysfunction precedes or is a consequence of biofilm formation. Biofilm formation has also been associated with the upregulation of mucin related genes, namely MUC5AC in goblet cells and MUC5B in submucous glands collected from mucosa of CRS patients. This increased expression and overproduction of mucin may act to trap more inhaled microbes and this, in concert with stasis from disordered ciliary function, may give bacteria opportunity to adhere and begin the process of biofilm formation [26].

Another potential mechanism for biofilm pathogenicity is intracellular uptake of particular pathogens. Once intracellular, *S. aureus* undergoes phenotype switching secondary to transcriptional changes that alter its expression of genes related to evasion of host immune response and antibiotic resistance. CRS patients who demonstrate evidence of intracellular *s aureus* at time of functional endoscopic sinus surgery (FESS) are more likely to have delayed clinical and microbiological relapse post-operatively, suggesting that intracellular residence can serve as a nidus for infection in recurrent disease [27].

Biofilm formation also evokes significant pro-inflammatory changes in several ways. Cantero et al. found that healthy human sinonasal explants exposed to *S. aureus* biofilms upregulated the expression of genes involved in the Nod2/NF-kB pathway with downstream release of proinflammatory factors IL-6, IL-8, and the neutrophil chemoattractant CXCL2 [28]. *S. aureus* biofilms have also been associated with changes in the inflammasome pathway. Inflammasomes are cytoplasmic protein complexes with pattern recognition receptors that detect microbial products and activate caspase-1. Caspase-1 then elicits a downstream release of pro- inflammatory cytokines like IL-1B and IL-18. Jardaleza et al. found that CRSwNP mucosal samples positive for *S. aureus* biofilms demonstrated upregulation of genes involved in the inflammasome signaling pathway, potentially explaining the persistent inflammation characteristic of CRS [29]. This pro-inflammatory state also contributes to the higher degree of osteitis seen in mucosa underlying biofilms in CRS affected mucosa; inflamed bone may in turn act as a “depot” for inflammatory cytokines contributing to the vicious cycle [30]. These changes overall induce an inflammatory infiltrate in the subepithelium and lamina propria featuring lymphocytes, dendritic cells, eosinophils, and plasma cells as early as 2 weeks into the disease. This infiltrate is followed by biofilm adherence to the epithelial surface with resultant damage to mucociliary function hindering clearance of the primary infection. This mucociliary impairment in turn increases susceptibility to other opportunistic pathogens [23, 31, 32].

Clinical Implications of Biofilm Positive CRS

The clinical implications associated with biofilm formation in CRS have been extensively investigated. CRS patients with biofilm have more severe disease prior to intervention as evidenced by worse objective clinical indicators of disease [33, 34] and higher subjective symptom burden [34, 35]. Patients with biofilm positive CRS also report significantly worse outcomes in quality of life at 6 months post-FESS compared to their biofilm negative counterparts [36]. Additionally, biofilm positive CRS patients demonstrate significantly worse objective outcomes with more severe disease on pre- and post-operative nasal

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

endoscopy, more follow up visits and additional courses of antibiotics when compared to biofilm negative CRS patients [14]. The presence of biofilms in CRS is also independently associated with greater risk of recurrence [34] and revision surgery [36].

The clinical outcomes of biofilm associated CRS have been found to depend on the bacterial species involved in the disease process. *S. aureus* biofilms in particular are associated with more severely recalcitrant disease and patients with *S. aureus* biofilms demonstrate worse objective markers of inflammation and report poorer quality of life than patients with biofilms formed from other bacterial or fungal species[14, 37]. This phenomenon is potentially due to the severe inflammatory response to *S. aureus* superantigens.

The Superantigen Hypothesis

Mechanism of Superantigen Stimulation

Bacterial biofilms are an import source for eluting superantigens. Superantigens (SAgs) are exotoxins produced by bacteria in order to evade host immune response. SAgs confer virulence by short-circuiting normal antigen presentation leading to massive release of inflammatory cytokines [38, 39]. In addition, the host experiences massive T cell activation, expansion, and subsequent anergy as well as B cell activation [38, 39]. In effect, host immunity is debilitated, and pathogenic bacteria persist. While multiple bacterial species are known to secrete superantigenic exotoxins, *Staphylococcus aureus* enterotoxins (SAEs) elicit the most pronounced clinical manifestations, such as toxic shock syndrome, gastroenteritis, atopic dermatitis, asthma, and chronic rhinosinusitis with nasal polyps [40]. SAEs, which include staphylococcal enterotoxins A through U and toxic shock syndrome toxin (TSST-1), are a relatively well-studied family of SAgs that are related to the larger class of exotoxins, called thermal stable proteins [41, 42].

In normal physiology, antigen presenting cells (APCs) internalize and process non-self peptides before presenting them on their MHC II molecules to T cells via TCRs. SAEs, however, can bind directly in an unprocessed form to MHC II molecules outside of peptide-binding groove. Upon presentation to TCRs, SAEs cause a hyper-stimulation of T cells, with nearly 20–30% activation of host T cells, compared to the normal 0.001%−0.001% [43, 44]. Given this robust effect, SAEs are capable of inducing clinical symptoms at concentrations as low as 0.1 pg/ml [45] Interestingly, SAgs are known to specifically interact with only the beta chain in the variable region of TCRs (Vβ), and each SAg interacts with only a subset of Vβ motifs [46]. Therefore, SAg-dependent T cell stimulation leads to characteristic expansion of oligoclonal T cells with specific Vβ signatures [47–49]. While optimal interaction of SAg-MHC II complex with TCRs involves co-stimulatory signals B7 and CD28 on APCs and T cells respectively, some SAgs are also known to stimulate TCRs directly in an MHC II-independent manner [50]. However, this mode of stimulation does not yield as strong of a T cell response.

Staphylococcal Superantigens in CRS

Studies have shown a link between superantigen-stimulated immune response and the development of CRS. It is established that superantigens, particularly SAEs, tend to promote

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

a Th2 immune response that favors eosinophilic inflammation found in CRSwNP, rather than a Th1 response found in CRSsNP [51, 52]. Initial data implicating SAEs in CRS demonstrated higher *S. aureus* culture-positive rates in patients with CRSwNP (45.4–71%) compared to CRSsNP (7.6%−42.8%) and healthy controls (0%−27.3%) [53–57]. Likewise, numerous studies have shown that SAEs were directly detectable by immunoassay in patients with CRSwNP [40, 55, 58–60]. However, other studies have shown conflicting data in which the association of *S. aureus* and SAEs with CRS was not found [56, 61]. It is well known that even healthy individuals are commonly carriers of *S. aureus* in the nose and sinuses [62]; moreover, the aforementioned studies showed that not every patient with CRSwNP was colonized. To address conflicting data, a 2014 meta-analysis of 340 cases concluded that four studies collectively showed an overall significantly greater association between CRSwNP and positive *S. aureus* nasal cultures (OR 4.85, 95 % Cl 1.80–13.05, $p = 0.002$) [63]. Data from another five studies in the same meta-analysis also demonstrated significant association between CRSwNP and directly measured SAEs (OR 12.07, 95 % Cl 4.57–31.90, $p < 0.00001$) [63]. While meta-analysis is limited by variation in study design and patient populations, the data do support that *S. aureus* infection and SAEs are found more often in patients with CRSwNP.

Research interest has since turned toward elucidating the underlying mechanism whereby SAEs drive Th2 inflammation observed in CRSwNP. Patou et al. first demonstrated using multiplex immunoassay that staphylococcal enterotoxin B (SEB) stimulates cultured nasal polyps to secrete a predominately type-2 cytokines (i.e., IL-2, IL-4, IL-5, IL-10, IL-13) [52]. Li et al., recently showed that presence of *S. aureus* reduced levels of Th1-promoking cytokine, IP-10, thereby contributing to Th2-bias [64]. Superantigens also stimulate local B cell immunoglobulin class switching and production. Numerous studies have demonstrated elevated IgE antibodies specific to SAEs in CRSwNP compared to CRSsNP and healthy controls, independent of atopy [53, 60, 65–67]. In addition, Van Zele et al. reported a rate of SAE-IgEs as high as 80% in a subgroup of CRSwNP patients with AERD and asthma, compared to 28% in CRSwNP lacking such comorbid lower-airway disease, 6% in CRSsNP, and 15% in healthy controls [68]. Levels of IgEs specific to SAEs also correlated with IL-5, eotaxin, eosinophilic cationic protein, which are responsible for driving eosinophilic infiltrate characteristic of the aberrant mucosa found in CRSwNP [69].

Emerging Treatments

The recurrent and recalcitrant nature of biofilm associated CRS and its significant impact on quality of life have recently prompted a focus on the search for effective anti-biofilm therapies. Here we summarize the most recent developments in potential treatment strategies.

The antibiotic resistance conferred by the biofilm state poses a challenge for systemic antibiotic penetration. Recently macrolides have been a focus of potential treatment given their ability to impair biofilm formation by interfering with quorum sensing. In a randomized controlled trial (RCT) by Tatar et al., 32 CRSwNP patients on an 8 week course of oral clarithromycin therapy prior to undergoing FESS were randomized to receive either concomitant topical mometasone furoate or oral macrolide therapy alone. Pre- and post-

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

treatment nasal tissue samples were compared for biofilm density and extension on SEM using a quantitative grading scale (range = 1–3). While overall biofilm prevalence decreased with treatment in both groups (75% vs 43.8%), and post-treatment grade was significantly decreased in both groups (median difference = 1.0, $p < 0.01$), no additional benefit was found for adding intranasal steroid to oral macrolide therapy in CRSwNP [11]. In a similar RCT, Korkmaz et al. compared the efficacy of intranasal steroid as monotherapy versus combined with low-dose oral macrolide in 34 CRSwNP patients [70]. The combined macrolide and intranasal steroid group had significant reduction of biofilm compared to control but the intranasal steroid group alone did not. Despite these studies suggesting a possible role for macrolides in eradicating biofilms, a recent meta-analysis found limited evidence to support macrolide efficacy in CRS. While oral macrolides can interfere with biofilm formation, penetration into an established biofilm may prove difficult and might account for the discrepancies in efficacy.

Topical antibiotic formulations have been a focus of recent research due to their ability to locally deliver higher concentrations of antibiotic therapy with limited systemic absorption. In a prospective trial Ezzat et al. investigated the efficacy of topical ofloxacin in 10 patients with refractory CRS and evidence of biofilm 6 months post-FESS. After 12 weeks of topical ofloxacin treatment there were no reported side effects and 80% of the study group demonstrated complete eradication of biofilm on SEM. The remaining 20% had reduced number of inflammatory cells, restoration of ciliary architecture, and only scattered areas of thin biofilm [71]. Other studies, however, have demonstrated limited efficacy of topical antibiotics like tobramycin and mupirocin and more large scale RCTs are needed before a recommendation for or against topical antibiotics can be reached [72–75].

Sinusitis is one of the most common indications for outpatient antibiotic prescription in adults, accounting for approximately 15–21% of all antibiotic prescriptions in the United States [76]. Finding novel non-antibiotic treatment modalities for CRS is of special importance given the growing concern for antibiotic resistance. Cirkovic et al. performed a prospective study to analyze the effects of intranasal steroids (mometasone furoate and fluticasone proprionate) and intranasal saline irrigation (isotonic and hypertonic) on the biofilm forming capacity of bacterial strains isolated from CRSwNP patients [77]. The effect of treatment was dependent on both the bacterial species and biofilm mass. All four treatment modalities resulted in a decrease in biofilm density but the greatest reduction of biofilm density was observed for *S. aureus*, *P. aeruginosa* and *S. pneumoniae* ($p < 0.01$, $p < 0.05$ and $p < 0.05$ respectively). Treatment effects were also more pronounced with bacterial strains that produced greater biofilm mass. These results demonstrate a need for directed antibiofilm treatment depending on the identity and potency of the microbial strain.

One such potential species specific treatment is a chimeric protein, P128, which combines a cell- wall binding domain specific to Staphylococcal species, and a phage derived hydrolase domain capable of breaking down peptidoglycan based cell walls. Biofilms formed by methicillin sensitive and methicillin resistant *S. aureus* (MSSA and MSRA) clinical isolates from CRS patients and a reference strain of S. aureus were incubated with varying concentrations of P128 ranging from 1.56–50 μg/mL and assessed for persistence after treatment. Biofilm mass was found to be significantly reduced in all *S. aureus* isolates at

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

P128 concentrates greater than 25 μg/mL compared to 0.9% saline control ($p < 0.05$). Given its action against virulent and resistant strains of S. aureus P128 has promising implications for the most severely recalcitrant CRS cases and antimicrobial stewardship.

Manuka honey (MH) includes high levels of a phenol compound, methylglyoxal (MGO) which has shown strong bactericidal activity against *S. aureus* and *P. aeruginosa* biofilms[78]. In a sheep model of sinusitis, MH augmented with additional MGO was found to significantly reduce biofilm mass at MGO concentrations of 1.8 mg/mL (0.676 ± 0.079 $\mu m^3/\mu m^2$ vs 0.114 ± 0.033 $\mu m^3/\mu m^2$, $p = 0.001$) and 3.6 mg/mL (0.608 ± 0.110 $\mu m^3/\mu m^2$ vs 0.316 ± 0.197 $\mu m^3/\mu m^2$, $p = 0.015$) when compared with saline flushes. However, when administered alone, MGO caused severe sinus inflammation and squamous metaplasia of the respiratory epithelium although this effect was not seen when MGO was administered in combination with MH [79]. Clinical trials assessing the safety and efficacy of MH/MGO in patients with biofilm positive CRS are needed.

Another naturally occurring compound with antibiofilm activity is xylitol - a five-carbon sugar alcohol. Jain et al. demonstrated that 5% and 10% solutions of xylitol significantly reduced S. epidermidis biofilm when compared to saline control ($\Delta 1.1 \pm 0.1$, $P<0.001$ and $\Delta 1.0 \pm 0.1$, $P<0.001$, respectively) but no significant difference was seen in biofilm reducing capacity for *s aureus* or *P. aeruginosa* biofilms compared to control. This result again demonstrates the complexity of determining the optimal treatment and management of biofilm positive CRS given that clinical behavior and therapeutic response differ based on the biofilm forming species.

Antimicrobial photodynamic therapy (aPDT) harnesses the power of oxygen free radicals inducing the perforation of bacterial cell membranes and allowing the penetration of photoreactive dyes. Once activated by laser light, these dyes induce photodamage to inner organelles and promote apoptosis. In a silicone model of the human maxillary sinus inoculated with MRSA and antibiotic resistant *P. aeruginosa*, Biel et al. demonstrated a greater than 99.9% reduction of biofilms grown from each species after a single treatment of aPDT [80]. aDPT has also demonstrated efficacy in preventing surgical site infections when used in pre-operative decolonization therapy in conjunction with chlorhexidine body wipes, in the treatment of chronic periodontitis, and in reducing biofilm formation on orthopedic prosthetic devices [81–83]. aPDT does not cause histologic damage to the underlying respiratory mucosa and appears to be a promising and viable option for biofilm eradication, especially in antibiotic resistant cases [84].

Recently, the use of ultrasound to physically disrupt established biofilms has been evaluated in two in vitro studies. Karosi et al. examined the efficacy of biofilm disruption using low-frequency ultrasound (LFU) on nasal polyps isolated from 10 CRSwNP patients. In LFU untreated polyps, the average biofilm thickness was 17.3 pm while polyps in the treated condition demonstrated total absence of bacterial biofilm ($p < 0.001$). The number of inflammatory cells present in the subepithelial layer was also significantly reduced in the LFU treated versus untreated group ($p < 0.001$). In a double blind RCT, researchers assessed the impact of LFU on subjective symptom severity for 60 CRS patients after 10 treatment sessions. Patients were randomized to LFU using either normal ultrasound transmission gel

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

or erythromycin ointment applied to the superficial facial skin overlying the maxillary and frontal sinuses[85]. Patients in both conditions reported significantly improved post-treatment symptom scores with large effect sizes (LFU d=1.36, LFU + erythromycin d=2.15). The percent improvement in pre- to post-treatment scores was significantly higher in the LFU plus erythromycin condition than the LFU alone condition (67.2 ± 23.7 vs 49.3 ± 37.2,p=0.03). The superior symptom relief in the erythromycin condition is thought to be from increased delivery of erythromycin to target tissues via synergistic ultrasound vibrations and increased blood flow in the skin overlying target tissues.

Future directions

Biofilms

A growing body of evidence implicates the role of biofilms in the pathophysiology of recurrent and recalcitrant CRS. Future studies exploring novel detection measures and antibiofilm treatments are needed. Non-invasive identification methods would allow for earlier and easier detection of biofilms and objective assessment of pre- and post-treatment changes. Poly-A- acetylglucosamine (PNAG) is a polysaccharide produced by *S. aureus* that is critical to biofilm formation that can serve as target for both *S. aureus* biofilm detection and disruption. Foremen et al. have developed a non-invasive immunofluorescence protocol that detects *S. aureus* biofilms in CRS patients using PNAG with similar accuracy to invasive methods [86]. Given that alterations in bitter and sweet taste receptors, like T2R38, can increase susceptibility to biofilm formation, bitter and sweet receptor sensitivity as assessed by a simple in-office taste test can serve to stratify patients most at risk for biofilm formation, and identify patients for whom T2R pathway agonists may be beneficial.

Future studies should also make every effort to stratify patients by CRS endotype given that CRSwNP and CRSsNP are heterogeneous disease processes with significantly different inflammatory profiles. In addition, the majority of treatment related studies have focused on *S. aureus* given its association with biofilm formation and severe CRS. However, it is clear that other bacterial species capable of forming biofilms demonstrate differential clinical behavior and therapeutic response.

Superantigens

The exact mechanisms by which superantigens perturb the immune system in CRSwNP is still being elucidated. Multiple studies have shown that distinct Vβ signatures are produced when host T cells undergo SAE-mediated stimulation in the setting of CRSwNP [49, 87, 88], and Tripathi et al. reported 58% of CRSwNP had detectable signatures Vβ clonal expansions of T cells [49]. In an era of more granular endotyping of CRS, understanding how these clonal expansions may serve as methods of immunophenotyping will add value.

In addition, further understanding host characteristics is key. Across most studies, data demonstrate that only half of patients with CRSwNP have detectable SAEs; therefore, host factors may be underlying SAE sensitivity [89, 90]. Recent investigation is underway examining variations in host HLAs, Vβ chains, and various forms of immunocompromised states. For example, hosts' abilities to rid pathogenic bacteria may be related not only to

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

SAE-eluting biofilms as discussed above, but also to intracellular residing *S. aureus* producing SAgs, which recent studies have identified by immunohistochemistry [91] and PNA-FISH [51].

New evidence also has implicated SAEs in influencing eicosanoids, which are inflammatory molecules generated from the metabolism of arachidonic acid. Involvement of the eicosanoid pathway was first suggested in patients with lower respiratory disease presenting with aspirin intolerance [92]. More recently, eicosanoids such as leukotrienes and pro-inflammatory leukotrienes have been known to be downstream of superantigens in the setting of Th2 upper airway disease [93–95]. Furthermore, Murawat et al. demonstrated that SAEs cause cytokine secretion in nasal polyps via phospholipase A2, the first and key regulatory enzyme in metabolizing eicosanoids [96]. Further investigation will hopefully shed increasing light on how these molecules are contributing.

Conclusions

CRS is a complex and multifactorial disease with a still unclear etiology that includes inflammatory, infectious, host and environmental factors. Two emerging areas of research of recent interest explore the potential role for biofilms and superantigens in the pathophysiology of CRS. A growing body of evidence suggests both entities play potentially intersecting roles in the development of recalcitrant disease. Despite the discovery of several novel, hypothesis driven, antibiofilm therapeutics, the optimal means of management for biofilm and superantigen associated CRS remains unclear. As our understanding of the role that biofilms and superantigens play in CRS deepens, we will be able to target the specific underlying causes of persistent CRS with species and mechanism specific multimodal treatments.

Acknowledgments

Funding: This work was supported by NIH grants R01DC013588 (to NAC.), R01DC013588– 04S2 (to IWM) and TL1R001880 (to NNP)

References

Papers of particular interest, published recently, have been highlighted as:

* Of importance

1. Rudmik L (2017) Economics of Chronic Rhinosinusitis. Curr Allergy Asthma Rep 17:20 [PubMed: 28337570]
2. Gliklich RE, Metson R (1995) The health impact of chronic sinusitis in patients seeking otolaryngologic care. Otolaryngol Head Neck Surg 113:104–9 [PubMed: 7603703]
3. Dennis SK, Lam K, Luong A (2016) A Review of Classification Schemes for Chronic Rhinosinusitis with Nasal Polyposis Endotypes. Laryngoscope Investig Otolaryngol 1:130–134
4. Donlan RM, Costerton JW (2002) Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 15:167–93 [PubMed: 11932229]
5. Donlan RM (2002) Biofilms: microbial life on surfaces. Emerg Infect Dis 8:881–90 [PubMed: 12194761]
6. Gilbert P, Das J (1997) BIOFILM SUSCEPTIBILITY TO ANTIMICROBIALS. Adv Dent Res U 160–167

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

7. Ramakrishnan Y, Shields RC, Elbadawey MR, Wilson JA (2015) Biofilms in chronic rhinosinusitis: what is new and where next? J Laryngol Otol 129:744–751 [PubMed: 26120023]
8. Boase S, Foreman A, Cleland E, Tan L, Melton-Kreft R, Pant H, Hu FZ, Ehrlich GD, Wormald P-J (2013) The microbiome of chronic rhinosinusitis: culture, molecular diagnostics and biofilm detection. BMC Infect Dis 13:210 [PubMed: 23656607]
9. Chole RA, Faddis BT (2003) Anatomical Evidence of Microbial Biofilms in Tonsillar Tissues. Arch Otolaryngol Neck Surg 129:634
10. Cryer J, Schipor I, Perl off JR, Palmer JN (2004) Evidence of Bacterial Biofilms in Human Chronic Sinusitis. ORL 66:155–158 [PubMed: 15316237]
11. Tatar EC, Tatar I, Ocal B, Korkmaz H, Saylam G, Ozdek A, Qelik HH (2012) Prevalence of Biofilms and Their Response to Medical Treatment in Chronic Rhinosinusitis without Polyps. Otolaryngol Neck Surg 146:669–675
12. Foreman A, Psaltis AJ, Tan LW, Wormald P-J (2010) Characterization of bacterial and fungal biofilms in chronic rhinosinusitis. Allergy Rhinol 1:10–10
13. Boase S, Jervis-Bardy J, Cleland E, Pant H, Tan L, Wormald P-J (2013) Bacterial-induced epithelial damage promotes fungal biofilm formation in a sheep model of sinusitis. Int Forum Allergy Rhinol 3:341–348 [PubMed: 23307805]
14. Singhal D, Psaltis AJ, Foreman A, Wormald P-J (2010) The impact of biofilms on outcomes after endoscopic sinus surgery. Am J Rhinol Allergy 24:169–174 [PubMed: 20537281]
15. Lee RJ, Cohen NA (2014) Bitter and sweet taste receptors in the respiratory epithelium in health and disease. J Mol Med (Berl) 92:1235–44 [PubMed: 25391251]
16. Zhang Y, Hoon MA, Chandrashekar J, Mueller KL, Cook B, Wu D, Zuker CS, Ryba NJP (2003) Coding of Sweet, Bitter, and Umami Tastes. Cell 112:293–301 [PubMed: 12581520]
17. Laffitte A, Neiers F, Briand L (2014) Functional roles of the sweet taste receptor in oral and extraoral tissues. Curr Opin Clin Nutr Metab Care 17:379–85 [PubMed: 24763065]
18. Lee RJ, Xiong G, Kofonow JM, et al. (2012) T2R38 taste receptor polymorphisms underlie susceptibility to upper respiratory infection. J Clin Invest 122:4145–4159 [PubMed: 23041624]
19. Lee RJ, Chen B, Redding KM, Margolskee RF, Cohen NA (2014) Mouse nasal epithelial innate immune responses to *Pseudomonas aeruginosa* quorum-sensing molecules require taste signaling components. Innate Immun 20:606–617 [PubMed: 24045336]
20. Meyerhof W, Batram C, Kuhn C, Brockhoff A, Chudoba E, Bufe B, Appendino G, Behrens M (2010) The Molecular Receptive Ranges of Human TAS2R Bitter Taste Receptors. Chem Senses 35:157–170 [PubMed: 20022913]
21. Bufe B, Breslin PAS, Kuhn C, Reed DR, Tharp CD, Slack JP, Kim U-K, Drayna D, Meyerhof W (2005) The Molecular Basis of Individual Differences in Phenylthiocarbamide and Propylthiouracil Bitterness Perception. Curr Biol 15:322–327 [PubMed: 15723792]
22. Workman AD, Brooks SG, Kohanski MA, et al. (2017) Bitter and sweet taste tests are reflective of disease status in chronic rhinosinusitis. J Allergy Clin Immunol Pract. doi: 10.1016/jjaip.2017.09.014
23. Jia M, Chen Z, Du X, Guo Y, Sun T, Zhao X (2014) A simple animal model of Staphylococcus aureus biofilm in sinusitis. Am J Rhinol Allergy 28:115–119
24. Dlugaszewska J, Leszczynska M, Lenkowski M, Tatarska A, Pastusiak T, Szyfter W (2016) The pathophysiological role of bacterial biofilms in chronic sinusitis. Eur Arch Otorhinolaryngol 273:1989–94 [PubMed: 26024693]
25. Galli J, Calo L, Ardito F, Imperiali M, Bassotti E, Cesare Passali G, La Torre G, Paludetti G, Fadda G (2008) Damage to Ciliated Epithelium in Chronic Rhinosinusitis: What Is the Role of Bacterial Biofilms? Ann Otol Rhinol Laryngol 117:902–908 [PubMed: 19140536]
26. Mao Y-J, Chen H-H, Wang B, Liu X, Xiong G-Y (2015) Increased expression of MUC5AC and MUC5B promoting bacterial biofilm formation in chronic rhinosinusitis patients. Auris Nasus Larynx 42:294–298 [PubMed: 25638393]
27. Tan NC-W, Foreman A, Jardeleza C, Douglas R, Vreugde S, Wormald P-J (2013) Intracellular *Staphylococcus aureus* : the Trojan horse of recalcitrant chronic rhinosinusitis? Int Forum Allergy Rhinol 3:261–266 [PubMed: 23423994]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

28. Cantero D, Cooksley C, Bassiouni A, Wormald P-J, Vreugde S (2013) Staphylococcus aureus biofilm activates the nucleotide-binding oligomerization domain containing 2 (Nod2) pathway and proinflammatory factors on a human sinonasal explant model. Int Forum Allergy Rhinol 3:877–884 [PubMed: 24039208]
29. Jardeleza C, Miljkovic D, Baker L, et al. (2013) Inflammasome gene expression alterations in Staphylococcus aureus biofilm-associated chronic rhinosinusitis. Rhinology 51:315–22 [PubMed: 24260763]
30. Dong D, Yulin Z, Xiao W, Hongyan Z, Jia L, Yan X, Jia W (2014) Correlation between bacterial biofilms and osteitis in patients with chronic rhinosinusitis. Laryngoscope 124:1071–1077 [PubMed: 24114791]
31. Karosi T, Csomor P, Hegyi Z, Sziklai I (2013) The presence of CD209 expressing dendritic cells correlates with biofilm positivity in chronic rhinosinusitis with nasal polyposis. Eur Arch Oto-Rhino-Laryngology 270:2455–2463
32. Arjomandi H, Glide J, Zhu S, Delaney S, Hochstim C, Mazhar K, Wrobel B, Markarian A, Masood R, Rice D (2013) Relationship of eosinophils and plasma cells to biofilm in chronic rhinosinusitis. Am J Rhinol Allergy 27:85–90
33. Wang X, Dong D, Cheng J, Fan X, Zhao Y (2015) Relationship between biofilms and clinical features in patients with sinus fungal ball. Eur Arch Oto-Rhino-Laryngology 272:2363–2369
34. Glowacki R, Tomaszewski KA, Strek P, Tomaszewska IM, Zgórska-Ś wierzy K, Markiewicz B, Skladzień J (2014) The influence of bacterial biofilm on the clinical outcome of chronic rhinosinusitis: a prospective, double-blind, scanning electron microscopy study. Eur Arch Oto-Rhino-Laryngology 271:1015–1021
35. Zhang Z, Adappa ND, Chiu AG, Doghramji LJ, Cohen NA, Palmer IN (2015) Biofilmforming bacteria and quality of life improvement after sinus surgery. Int Forum Allergy Rhinol 5:643–9 [PubMed: 25763837]
36. Zhang Z, Linkin DR, Finkelman BS, O'Malley BW, Thaler ER, Doghramji L, Kennedy DW, Cohen NA, Palmer IN (2011) Asthma and biofilm-forming bacteria are independently associated with revision sinus surgeries for chronic rhinosinusitis. J Allergy Clin Immunol 128:221–223.el [PubMed: 21439623]
37. Singhal D, Foreman A, Jervis-Bardy J, Bardy J-J, Wormald P-J (2011) Staphylococcus aureus biofilms: Nemesis of endoscopic sinus surgery. Laryngoscope 121:1578–83 [PubMed: 21647904]
38. Watson ARO, Janik DK, Lee WT (2012) Superantigen-induced CD4 memory T cell anergy. I. Staphylococcal enterotoxin B induces Fyn-mediated negative signaling. Cell Immunol 276:16–25 [PubMed: 22386537]
39. William T Lee DKJ (2015) Staphylococcal Enterotoxin B (SEB) Induces Memory CD4 T Cell Anergy in vivo and Impairs Recall Immunity to Unrelated Antigens. J Clin Cell Immunol, doi: 10.4172/2155-9899.1000346
40. Seiberling KA, Grammer LC, Kern RC (2009) Chronic rhinosinusitis and superantigens. Rhinol Facial Plast Surg 38:231–239
41. Spaulding AR, Salgado-Pabó n W, Kohler PL, Horswill AR, Leung DYM, Schlievert PM (2013) Staphylococcal and streptococcal superantigen exotoxins. Clin Microbiol Rev 26:422–447 [PubMed: 23824366]
42. Krakauer T, Stiles BG (2013) The staphylococcal enterotoxin (SE) family: SEB and siblings. Virulence, doi: 10.4161/viru.23905
43. Proft T, Fraser JD (2003) Bacterial superantigens. Clin Exp Immunol 133:299–306 [PubMed: 12930353]
44. Fraser JD, Proft T (2008) The bacterial superantigen and superantigen-like proteins. Immunol Rev 225:226–243 [PubMed: 18837785]
45. Bohach GA, Fast DJ, Nelson RD, Schlievert PM (1990) Staphylococcal and streptococcal pyrogenic toxins involved in toxic shock syndrome and related illnesses. Crit Rev Microbiol 17:251–272 [PubMed: 2206394]
46. Kappler J, Kotzin B, Herron L, Gelfand EW, Bigler RD, Boylston a, Carrel S, Posnett DN, Choi Y, Marrack P(1989) V beta-specific stimulation of human T cells by staphylococcal toxins. Science 244:811–813 [PubMed: 2524876]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

47. Llewelyn M, Sriskandan S, Terrazzini N, Cohen J, Altmann DM (2006) The TCR Vβ signature of bacterial superantigens spreads with stimulus strength. Int Immunol 18:1433–1441 [PubMed: 16893924]
48. Ferry T, Thomas D, Perpoint T, Lina G, Monneret G, Mohammedi I, Chidiac C, Peyramond D, Vandenesch F, Etienne J (2008) Analysis of superantigenic toxin Vβ T-cell signatures produced during cases of staphylococcal toxic shock syndrome and septic shock. Clin Microbiol Infect 14:546–554 [PubMed: 18373692]
49. Conley DB, Tripathi A, Seiberling KA, Schleimer RP, Suh LA, Harris K, Paniagua MC, Grammer LC, Kern RC (2006) Superantigens and chronic rhinosinusitis: skewing of T- cell receptor V beta-distributions in polyp-derived CD4+ and CD8+ T cells. Am J Rhinol 20:534–539 [PubMed: 17063750]
50. Thibodeau J, Dohlsten M, Cloutier I, Lavoie PM, Bjork P, Michel F, Leveille C, Mourad W, Kalland T, Sekaly RP (1997) Molecular characterization and role in T cell activation of staphylococcal enterotoxin A binding to the HLA-DR alpha-chain. JImmunol 158:3698–3704 [PubMed: 9103433]
51. Sachse F, Becker K, Von Eiff C, Metze D, Rudack C (2010) Staphylococcus aureus invades the epithelium in nasal polyposis and induces IL-6 in nasal epithelial cells in vitro. Allergy Eur J Allergy Clin Immunol 65:1430–1437
52. Patou J, Gevaert P, Van Zele T, Holtappels G, van Cauwenberge P, Bachert C (2008) Staphylococcus aureus enterotoxin B, protein A, and lipoteichoic acid stimulations in nasal polyps. J Allergy Clin Immunol 121:110–115 [PubMed: 17980412]
53. Foreman A, Holtappels G, Psaltis AJ, Jervis-Bardy J, Field J, Wormald P-J, Bachert C (2011) Adaptive immune responses in Staphylococcus aureus biofilm-associated chronic rhinosinusitis. Allergy 66:1449–1456 [PubMed: 21834937]
54. Gevaert P, Holtappels G, Johansson SGO, Cuvelier C, Van Cauwenberge P, Bachert C (2005) Organization of secondary lymphoid tissue and local IgE formation to Staphylococcus aureus enterotoxins in nasal polyp tissue. Allergy Eur J Allergy Clin Immunol 60:71–79
55. El Fiky LM, Khamis N, El Din Mostafa D, Adly AM (2009) Staphylococcal infection and toxin production in chronic rhinosinusitis. AmJRhinolAllergy 23:264–267
56. Wang M, Zhang D, Zhang H ping, Wang Z, Chen X, Jian J fen (2006) [A primary study about the correlation between superantigens produced by Staphylococcus aureus and chronic rhinosinusitis with nasal polyps]. Lin Chuang Er Bi Yan Hou Ke Za Zhi 20:914–6 [PubMed: 17225516]
57. Bachert C, Gevaert P, Zhang N, van Zele T, Perez-Novo C (2007) Role of Staphylococcal Superantigens in Airway Disease. Superantigens and Superallergens 93:214–236
58. Guven M, Karabay O, Akidil O, Yilmaz MS, Yildirim M (2013) Detection of staphylococcal exotoxins in antrochoanal polyps and chronic rhinosinusitis with nasal polyps. Otolaryngol - Head Neck Surg (United States) 148:302–307
59. Wang M, Shi P, Chen B, Zhang H, Jian J, Chen X, Wang Z, Zhang D (2008) The Role of Superantigens in Chronic Rhinosinusitis with Nasal Polyps. ORL 70:97–103 [PubMed: 18408407]
60. Seon TK, Seung WC, Joo HJ, Jong SH, II GK (2011) Association of T cells and eosinophils with Staphylococcus aureus exotoxin A and toxic shock syndrome toxin 1 in nasal polyps. Am J Rhinol Allergy 25:19–24 [PubMed: 21711967]
61. Ramakrishnan VR, Feazel LM, Abrass LJ, Frank DN (2013) Prevalence and abundance of Staphylococcus aureus in the middle meatus of patients with chronic rhinosinusitis, nasal polyps, and asthma. Int Forum Allergy Rhinol 3:267–271 [PubMed: 23055295]
62. Zhang N, Gevaert P, van Zele T, Perez-Novo C, Patou J, Holtappels G, van Cauwenberge P, Bachert C (2005) An update on the impact of Staphylococcus aureus enterotoxins in chronic sinusitis with nasal polyposis. Rhinology 43:162–168 [PubMed: 16218508]
63. Ou J, Wang J, Xu Y, Tao Z zhang, Kong Y gang, Chen S ming, Shi W dan (2014) Staphylococcus aureus superantigens are associated with chronic rhinosinusitis with nasal polyps: a meta-analysis. Eur Arch Oto-Rhino-Laryngology 271:2729–36
64. Li Z, Levast B, Madrenas J (2017) *Staphylococcus aureus* Downregulates IP-10 Production and Prevents Th1 Cell Recruitment. J Immunol 198:1865–1874 [PubMed: 28122962]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

65. Conley DB, Tripathi A, Ditto AM, Reid K, Grammer LC, Kern RC (2004) Chronic sinusitis with nasal polyps: Staphylococcal exotoxin immunoglobulin E and cellular inflammation. Am J Rhinol 18:273–278 [PubMed: 15586797]
66. Tripathi A, Conley DB, Grammer LC, Ditto AM, Lowery MM, Seiberling KA, Yarnold PA, Zeifer B, Kern RC (2004) Immunoglobulin E to staphylococcal and streptococcal toxins in patients with chronic sinusitis/nasal polyposis. Laryngoscope 114:1822–6 [PubMed: 15454779]
67. Cui X, Miao J, Lu H, Qi Q, Chen X, Xu J, Lin Z, Chen Z, Yin M, Cheng L (2015) Serum levels of specific IgE to Staphylococcus aureus enterotoxins in patients with chronic rhinosinusitis. Exp Ther Med 1523–1527 [PubMed: 25780463]
68. Van Zele T, Gevaert P, Watelet J-B, Claeys G, Holtappels G, Claeys C, van Cauwenberge P, Bachert C (2004) Staphylococcus aureus colonization and IgE antibody formation to enterotoxins is increased in nasal polyposis. J Allergy Clin Immunol 114:981–983 [PubMed: 15480349]
69. Wang JH, Kwon HJ, Jang YJ (2010) Staphylococcus aureus increases cytokine and matrix metalloproteinase expression in nasal mucosae of patients with chronic rhinosinusitis and nasal polyps. Am J Rhinol Allergy 24:422–427 [PubMed: 20961499]
70. Korkmaz H Ö cal B, Tatar EÇ, Tatar I, Ö zdek A, Saylam G, Çelik HH (2014) Biofilms in chronic rhinosinusitis with polyps: is eradication possible? Eur Arch Oto-Rhino- Laryngology 271:2695–2702
71. Ezzat WF, Fawaz SA, Rabie H, Hamdy TA, Shokry YA (2015) Effect of topical ofloxacin on bacterial biofilms in refractory post-sinus surgery rhino-sinusitis. Eur Arch Oto-Rhino-Laryngology 272:2355–2361
72. Chiu AG, Antunes MB, Palmer JN, Cohen NA (2007) Evaluation of the in vivo efficacy of topical tobramycin against Pseudomonas sinonasal biofilms. J Antimicrob Chemother 59:1130–1134 [PubMed: 17405780]
73. Lee JT, Chiu AG (2014) Topical anti-infective sinonasal irrigations: Update and literature review. Am J Rhinol Allergy 28:29–38 [PubMed: 24717876]
74. El Haj C, Murillo O, Ribera A, Garcia-Somoza D, Tubau F, Cabellos C, Cabo J, Ariza J (2017) The anti-biofilm effect of macrolides in a rat model of S. aureus foreign-body infection: Might it be of clinical relevance? Med Microbiol Immunol 206:31–39 [PubMed: 27639707]
75. Head K, Chong LY, Piromchai P, Hopkins C, Philpott C, Schilder AG, Burton MJ (2016) Systemic and topical antibiotics for chronic rhinosinusitis. Cochrane Database Syst Rev 4:CD011994 [PubMed: 27113482]
76. Smith SS, Evans CT, Tan BK, Chandra RK, Smith SB, Kern RC (2013) National burden of antibiotic use for adult rhinosinusitis. J Allergy Clin Immunol 132:1230–2 [PubMed: 23987794]
77. Cirkovic I, Pavlovic B, Bozic DD, Jotic A, Bakic L, Milovanovic J (2017) Antibiofilm effects of topical corticosteroids and intranasal saline in patients with chronic rhinosinusitis with nasal polyps depend on bacterial species and their biofilm-forming capacity. Eur Arch Oto-Rhino-Laryngology 274:1897–1903
78. Alandejani T, Marsan J, Ferris W, Slinger R, Chan F (2009) Effectiveness of honey on *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms. Otolaryngol Neck Surg 141:114–118
79. Paramasivan S, Drilling AJ, Jardeleza C, Jervis-Bardy J, Vreugde S, Wormald PJ (2014) Methylglyoxal-augmented manuka honey as a topical anti- *Staphylococcus aureus* biofilm agent: safety and efficacy in an in vivo model. Int Forum Allergy Rhinol 4:187–195 [PubMed: 24415444]
80. Biel MA, Pedigo L, Gibbs A, Loebel N (2013) Photodynamic therapy of antibiotic- resistant biofilms in a maxillary sinus model. Int Forum Allergy Rhinol 3:468–473 [PubMed: 23307793]
81. Bryce E, Wong T, Forrester L, Masri B, Jeske D, Barr K, Errico S, Roscoe D (2014) Nasal photodisinfection and chlorhexidine wipes decrease surgical site infections: a historical control study and propensity analysis. J Hosp Infect 88:89–95 [PubMed: 25171975]
82. Monzavi A, Chinipardaz Z, Mousavi M, Fekrazad R, Moslemi N, Azaripour A, Bagherpasand O, Chiniforush N (2016) Antimicrobial photodynamic therapy using diode laser activated indocyanine green as an adjunct in the treatment of chronic periodontitis: A randomized clinical trial. Photodiagnosis Photodyn Ther 14:93–97 [PubMed: 26921460]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

83. Vassena C, Fenu S, Giuliani F, Fantetti L, Roncucci G, Simonutti G, Romanò CL, De Francesco R, Drago L (2014) Photodynamic antibacterial and antibiofilm activity of RLP068/C1 against Staphylococcus aureus and Pseudomonas aeruginosa forming biofilms on prosthetic material. Int J Antimicrob Agents 44:47–55 [PubMed: 24933446]
84. Biel MA, Jones JW, Pedigo L, Gibbs A, Loebel N (2012) The effect of antimicrobial photodynamic therapy on human ciliated respiratory mucosa. Laryngoscope 122:2628–2631 [PubMed: 23070780]
85. Ansari NN, Naghdi S, Fathali M, Bartley J, Rastak MS (2015) A randomized clinical trial comparing pulsed ultrasound and erythromycin phonophoresis in the treatment of patients with chronic rhinosinusitis. Physiother Theory Pract 31:166–172 [PubMed: 25495769]
86. Foreman A, Jervis-Bardy J, Boase SJ, Tan L, Wormald P-J (2013) Noninvasive *Staphylococcus aureus* biofilm determination in chronic rhinosinusitis by detecting the exopolysaccharide matrix component poly- *N*-acetylglucosamine. Int Forum Allergy Rhinol 3:83–88 [PubMed: 23136110]
87. Bernstein JM, Kansal R (2005) Superantigen hypothesis for the early development of chronic hyperplastic sinusitis with massive nasal polyposis. Curr Opin Otolaryngol Head Neck Surg 13:39–44 [PubMed: 15654214]
88. Tripathi A, Kern R, Conley DB, Seiberling K, Klemens JC, Harris KE, Suh L, Huang J, Grammer LC (2005) Staphylococcal exotoxins and nasal polyposis: analysis of systemic and local responses. Am J Rhinol 19:327–333 [PubMed: 16171163]
89. Schubert MS (2001) A superantigen hypothesis for the pathogenesis of chronic hypertrophic rhinosinusitis, allergic fungal sinusitis, and related disorders. Ann Allergy, Asthma Immunol 87:181–188 [PubMed: 11570613]
90. Gabra N, Alromaih S, Endam LM, Brito RM, Lariviere F, Al-Mot S, Ledeist F, Desrosiers M (2014) Clinical features of cytotoxic CD8+ T-lymphocyte deficiency in chronic rhinosinusitis patients: A demographic and functional study. Int Forum Allergy Rhinol 4:495–501 [PubMed: 24639246]
91. Ou J, Bassiouni A, Drilling A, Psaltis AJ, Vreugde S, Wormald PJ (2017) The persistence of intracellular Staphylococcus aureus in the sinuses: a longitudinal study. Rhinology 55:305–311 [PubMed: 28687814]
92. Forster U, Strathmann S, Schafer D, Szczepek AJ, Olze H (2013) Eicosanoid imbalance correlates in vitro with the pattern of clinical symptoms of Samter´s triad. Rhinology 51:61–69 [PubMed: 23441313]
93. Roca-Ferrer J, Garcia-Garcia FJ, Pereda J, Perez-Gonzalez M, Pujols L, Alobid I, Mullol J, Picado C (2011) Reduced expression of COXs and production of prostaglandin E2 in patients with nasal polyps with or without aspirin-intolerant asthma. J Allergy Clin Immunol 128:66–72 [PubMed: 21397936]
94. Perez-Novo CA, Waeytens A, Claeys C, Cauwenberge P Van, Bachert C (2008) Staphylococcus aureus enterotoxin B regulates prostaglandin E2 synthesis, growth, and migration in nasal tissue fibroblasts. J Infect Dis 197:1036–43 [PubMed: 18419541]
95. Okano M, Fujiwara T, Haruna T, Kariya S, Makihara S, Higaki T, Nishizaki K (2009) Prostaglandin E2 suppresses staphylococcal enterotoxin-induced eosinophilia-associated cellular responses dominantly through an E-prostanoid 2-mediated pathway in nasal polyps. J Allergy Clin Immunol. doi: 10.1016/j.jaci.2009.01.047
96. Mruwat R, Kivity S, Landsberg R, Yedgar S, Langier S (2015) Phospholipase A2- dependent release of inflammatory cytokines by superantigen-stimulated nasal polyps of patients with chronic rhinosinusitis. Am J Rhinol Allergy 29:el22–el28