

Chronic rhinosinusitis pathogenesis

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There are a variety of medical conditions associated with chronic sinonasal inflammation, including chronic rhinosinusitis (CRS) and cystic fibrosis. In particular, CRS can be divided into 2 major subgroups based on whether nasal polyps are present or absent. Unfortunately, clinical treatment strategies for patients with chronic sinonasal inflammation are limited, in part because the underlying mechanisms contributing to disease pathology are heterogeneous and not entirely known. It is hypothesized that alterations in mucociliary clearance, abnormalities in the sinonasal epithelial cell barrier, and tissue remodeling all contribute to the chronic inflammatory and tissue-deforming processes characteristic of CRS. Additionally, the host innate and adaptive immune responses are also significantly activated and might be involved in pathogenesis. Recent advancements in the understanding of CRS pathogenesis are highlighted in this review, with special focus placed on the roles of epithelial cells and the host immune response in patients with cystic fibrosis, CRS without nasal polyps, or CRS with nasal polyps. (*J Allergy Clin Immunol* 2015;136:1442-53.)

Key words: Chronic rhinosinusitis, nasal polyps, mucociliary clearance, epithelial cells, inflammation, microbiome

Abbreviations used

ASL:	Airway surface liquid
CF:	Cystic fibrosis
CFTR:	Cystic fibrosis transmembrane conductance regulator
CRS:	Chronic rhinosinusitis
CRSsNP:	Chronic rhinosinusitis without nasal polyps
CRSwNP:	Chronic rhinosinusitis with nasal polyps
MCC:	Mucociliary clearance
NO:	Nitric oxide
NOS:	Nitric oxide synthase
PAMP:	Pathogen-associated molecular pattern
T2R:	Taste family type 2 receptor
Treg:	Regulatory T
UT:	Uncinate tissue

Chronic rhinosinusitis (CRS) is characterized by chronic inflammation of the sinonasal mucosa and clinically associated with sinus pressure, nasal congestion, rhinorrhea, and a decreased sense of smell persisting for greater than 12 weeks.¹ CRS can be subdivided into 2 major categories based on whether nasal polyps are present (chronic rhinosinusitis with nasal polyps [CRSwNP]) or absent (chronic rhinosinusitis without nasal polyps [CRSsNP]).² Although CRS is estimated to affect more than 10 million patients in the United States and leads to \$22 billion in total annual costs,^{1,3} there are other diseases, such as cystic fibrosis (CF), that also involve chronic sinonasal inflammation and nasal polyp formation that have important clinical implications. A better understanding of CRS pathogenesis is needed to advance the current diagnostic and treatment strategies available for affected patients.

THE SINONASAL MICROBIOME

Much like the gut, the sinonasal cavity has a resident flora that maintains an environment conducive to respiratory health. Substantial effort has recently been made using culture-independent techniques (ie, molecular diagnostics) to attempt to understand and define the microbial community or microbiome of the human sinonasal cavity in the healthy and diseased (CRS) states.⁴⁻¹⁰ No consistent patterns have emerged in the diseased state to implicate a specific organism or organisms as causative, but data suggest an imbalance (or dysbiosis) is found in patients with CRS with a decrease in microbial diversity. Another concept that has emerged is that the correct balance of microbes within the local microbiome might be immunomodulatory and that an imbalance shifts an important regulator of local inflammation.¹¹ Puzzles that remain include the existence of similar microbial species in

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Some of the research described in this review and effort directed towards writing the review was supported by USPHS grants R01DC013588, R21DC013886 (to N.A.C.), and R03DC013862 (to R.J.L.); National Institutes of Health grants T32 AI083216 and R01 AI104733 (to R.P.S.); the Ernest S. Bazley Foundation (to R.P.S.); the Chronic Rhinosinusitis Integrative Studies Program U19-AI106683 (to R.P.S.), and a philanthropic contribution from the RLG Foundation (to N.A.C.).

Disclosure of potential conflict of interest: R. J. Lee has received a grant from the National Institutes of Health (NIH). R. P. Schleimer has received grants from the NIH; has consultant arrangements with Intersect ENT, GlaxoSmithKline, Allakos, Aurasense, Merck, BioMarck, and Sanofi; and has stock/stock options with Allakos, Aurasense, and BioMarck. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication September 29, 2015; revised October 21, 2015; accepted for publication October 21, 2015.

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0091-6749/\$36.00

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<http://dx.doi.org/10.1016/j.jaci.2015.10.009>

Terms in boldface and italics are defined in the glossary on page 1443.

both healthy subjects and patients with CRS, although in different abundances⁸; the fact that traditional pathogenic microbes, such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Stenotrophomonas maltophilia*, and *Enterobacter* species, are also found in healthy cavities, although at lower abundances⁸; and how innate and adaptive immune mechanisms (described below) can discriminate between bacterial species to help set the nasal microbiome.

MUCOCILIARY CLEARANCE: THE FOUNDATION OF SINONASAL INNATE IMMUNITY

The upper airways play an important role in removing particulates and pathogens from inspired air through mucociliary clearance (MCC),¹² a specialized function unique to the airway epithelium. MCC is the primary physical defense of the respiratory tract, complementing the physical epithelial barrier (Fig 1). MCC relies on both mucus production and transport. The airway surface liquid (ASL) lining the respiratory tract consists of 2 layers. The top is an antimicrobial-rich mucus

“gel” formed by mucins produced by *goblet cells* and submucosal glands.¹³ Mucins are large thread-like glycoproteins¹⁴ with “sticky” carbohydrate side chains¹⁵ that can bind surface adhesins on microorganisms,¹⁵ including *Mycoplasma pneumoniae*,¹⁶ *H influenzae*,¹⁷ *M catarrhalis*,¹⁸ *Pseudomonas aeruginosa*,¹⁹ and *Pseudomonas cepacia*.²⁰ The mucus layer rests on top of a less-viscous fluid periciliary layer that surrounds the cilia of airway epithelial cells and allows them to beat rapidly (approximately 8-15 Hz). Membrane-tethered mucins on the apical membrane of ciliated cells can form a “lubricating” brush-like structure that keeps the mucus and PLC layers separate to facilitate MCC.²¹ Coordinated and directional ciliary beating (known as the metachronal wave²²) facilitates transport of debris-laden mucus through the sinonasal cavity to the oropharynx, where it is swallowed or expectorated.

MCC is regulated by small-molecule neurotransmitter (eg, adenosine triphosphate and acetylcholine) and neuropeptide (eg, vasoactive intestinal peptide and substance P) receptors that regulate mucus and fluid secretion²³ and ciliary beating,²⁴ as well as receptors for bacterial products²⁵ and mechanical stresses.²⁶

GLOSSARY

ACANTHOLYSIS: Disruption of intercellular connections, such as desmosomes, resulting in loss of cohesion between epithelial cells and either basement membrane or basal cells.

ACANTHOSIS: Abnormal but benign proliferation of the epithelium.

ADHERENS JUNCTIONS: A complex of cell adhesion proteins, including the transmembrane protein E-cadherin and the intracellular components p120-catenin, β -catenin, and α -catenin, which is also important in epithelial barrier function. These types of junctions are basal to tight junctions.

BACTERIAL CpG: A pathogen-associated molecular pattern that is abundant in microbial genomes but not vertebrate genomic DNAs and is recognized by Toll-like receptor 9.

CD4⁺ T CELLS: A subset of T cells that express CD4 and help orchestrate an immune response by secreting a variety of different cytokines and promoting B-cell activation.

CD8⁺ T CELLS: A subset of T cells that express CD8 and can destroy target cells through the production of cytotoxic granule proteins, such as granzyme B and perforin.

CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR (CFTR): An anion channel that conducts chloride and thiocyanate ions across epithelial cell membranes. Mutations in the gene encoding CFTR are associated with cystic fibrosis.

D-DIMER: Small protein fragment generated from the breakdown of fibrin.

DESMOSOMES: A complex of cell adhesion proteins, including desmoplakin, desmoglein, and plakoglobin, that is also important in epithelial barrier function.

FACTOR XIIa: A coagulation factor also known as Hageman factor that is responsible for stabilizing a fibrin clot.

FIBRIN: An important protein, also known as Factor Ia, that is the critical component within a blood clot on activation and cross-linking.

FLAGGRIN: An epidermal protein important in barrier function of the skin that is mutated in patients with some forms of atopic dermatitis.

GOBLET CELL: Modified simple columnar epithelial cell which functions to secrete mucus.

LPS: A major component of the outer membrane of gram-negative bacteria that elicits a strong inflammatory response by engaging Toll-like receptor 4. LPS is also referred to as endotoxin.

NADPH OXIDASE: An enzyme involved in the production of reactive oxygen species.

REACTIVE OXYGEN SPECIES (ROS): Chemically reactive molecules that are generated during the metabolism of oxygen. During times of stress, high levels of ROS can result in significant tissue damage.

REGULATORY T CELLS: A subset of CD4 T cells that are important in promoting immunologic tolerance and might express the transcription factor forkhead box protein 3 and/or secrete the suppressive cytokines TGF- β and IL-10.

S100 PROTEINS: A family of more than 20 different low-molecular-weight proteins that all share a similar conformational structure and play a role in a variety of cellular processes. S100A7 (also known as psoriasin) has antimicrobial properties and is important in cell differentiation. S100A8/9 (also known as calprotectin) also has antimicrobial properties and can bind to essential metals, such as calcium and zinc.

TIGHT JUNCTION: A complex of cell adhesion proteins, including occludin, claudin family proteins, and JAM family proteins, that is largely responsible for creating an intact epithelial cell barrier.

TISSUE PLASMINOGEN ACTIVATOR: A serine protease that is the major enzyme responsible for the breakdown of fibrin clots.

TOLL-LIKE RECEPTORS: A class of protein receptors that recognize different conserved molecules derived from pathogens. These receptors play a critical role in the activation of the innate immune response.

TYPE 1 INFLAMMATORY RESPONSE: A type of immune response characterized by production of inflammatory cytokines, such as IFN- γ and IL-12, which are important in cell-mediated immunity and phagocyte-dependent inflammation.

TYPE 2 INFLAMMATORY RESPONSE: A type of immune response characterized by production of the inflammatory cytokines IL-4, IL-5, and IL-13 that is important for the clearance of large extracellular pathogens. This type of inflammation is observed in patients with allergic disease.

TYPE 2 INNATE LYMPHOID CELLS: A type of innate immune cell that produces type 2 cytokines, including IL-5 and IL-13, and might be important in a variety of type 2 inflammatory responses.

TYPE I INTERFERONS: A subgroup of cytokines that play a predominant role in the host defense against viruses.

The Editors wish to acknowledge Kristina Bielewicz, MS, for preparing this glossary.

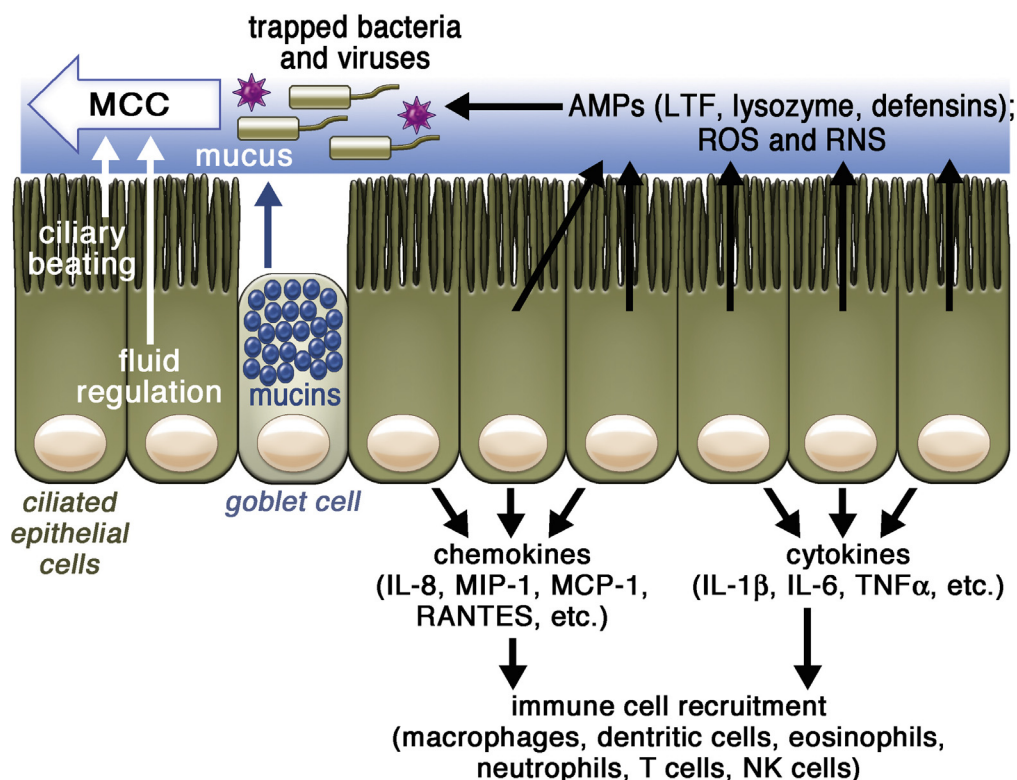


FIG 1. Overview of sinonasal innate immunity. In healthy tissue respiratory epithelial cells are linked by tight junctions to form a protective physical barrier. Inhaled pathogens, such as viruses, bacteria, and fungal spores, are trapped by airway mucus and then removed by means of MCC. Constant beating of cilia drives the pathogen-laden mucus toward the oropharynx, where it is then cleared out of the airway by means of expectoration or swallowing. MCC is further regulated by secretion of mucus, as well as ion and fluid transport, which controls mucus viscosity. Mucociliary transport is complemented by the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the production of antimicrobial peptides (AMPs). During more chronic exposure to pathogens, epithelial cells secrete cytokines to activate inflammatory pathways and recruit dedicated immune cells. LTF, Lactotransferrin; MCP-1, monocyte chemoattractant protein 1; MIP-1, macrophage inflammatory protein-1.

Although mechanisms in addition to MCC defend the airway (described below), the importance of MCC is illustrated by direct links between MCC defects and disease. In patients with CF, defects in the *cystic fibrosis transmembrane conductance regulator* (CFTR) anion channel result in impaired salt and water secretion,²³ as well as possibly enhanced absorption,²⁷ creating dehydrated mucus and impaired MCC. Patients with CF frequently have severe recurrent sinonasal infections²⁸ that might also seed or exacerbate lung infections. Additionally, levels of the epithelial anion transporter pendrin are increased in nasal polyps compared with those seen in uncinat tissue (UT) isolated from healthy control subjects.^{29,30} Upregulated pendrin expression in the airway has been linked to IL-4, IL-13, and IL-17A.³¹⁻³³ However, further studies are needed to investigate how pendrin contributes to MCC, epithelial dysfunction, and CRSwNP pathogenesis. Increased mucus production might also impair MCC. In patients with CRSwNP, Muc5AC levels were found to be increased in nasal polyps when compared with those in UT from patients with CRSsNP or healthy subjects.²⁹

Defects involving epithelial cell cilia can also affect MCC and contribute to chronic sinonasal inflammation. For example, in patients with primary ciliary dyskinesia, abnormal ciliary function, structure, or both result in impaired MCC and increased incidences of upper respiratory tract infections.³⁴ More commonly, however, acquired ciliary dysfunction occurs through

exposure to environmental or microbial toxins, as a secondary consequence of disease through exposure to inflammatory stimuli, or both.³⁵ Nonetheless, this likely contributes to pathogenesis of upper respiratory tract infections. Several pathogens produce compounds that impair ciliary motion, coordination, or both, including *H influenzae*, *S pneumoniae*, *Staphylococcus aureus*, *Aspergillus fumigatus*, and *P aeruginosa*.³⁶⁻³⁹ Hypoxia created by means of mucostasis or anatomic obstruction can also affect MCC by inhibiting ion transport⁴⁰ or promoting polypogenesis.⁴¹ Approaches designed to increase ciliary beating or enhance fluid secretion to thin mucus remain attractive therapeutic strategies for enhancing MCC in patients with CRS with impaired MCC. Additionally, high-volume, low-pressure sinonasal lavage has been demonstrated to be effective in mobilizing the copious secretions associated with CRS.⁴²

EPITHELIUM-DERIVED ANTIMICROBIAL PEPTIDES AND RADICALS

In addition to transporting mucus, sinonasal epithelial cells produce substances with direct antipathogen effects (Fig 1).^{43,44} These include well-characterized proteins, such as lysozyme, lactoferrin, antitrypsin, defensins, *S100 proteins*, and surfactants. Some are tonically secreted, but the expression of many is upregulated during infection.⁴⁵ Moreover, after epithelial

damage, concentrations of these proteins might increase further because of plasma extravasation.⁴⁶

Lysozyme is a small cationic protein secreted by submucosal glands.⁴⁷ Lysozyme catalyzes the breakdown of the β -1,4-glycosidic bonds between N-acetylmuramic acid and N-acetyl-D-glucosamine in the outer bacterial cell wall. Additionally, binding of lysozyme to bacterial cell walls facilitates phagocytosis by macrophages.⁴⁵ Lysozyme is most effective against gram-positive bacteria but also has effects against gram-negative bacteria^{48,49} and fungi.⁵⁰ Various studies have examined lysozyme in patients with CRS. However, controversy exists over whether levels are increased⁵¹ or decreased^{52,53} in patients with CRS. Lysozyme is produced by submucosal glands, which are diminished in nasal polyp tissue, and thus a decrease in lysozyme levels in polyp tissue might contribute to the variability of reported results.⁵⁴

Lactoferrin (also known as lactotransferrin) chelates and sequesters iron, which is important for bacterial and fungal metabolism.⁴⁵ Bacteria can also use iron to catalyze mucin degradation to help break through the protective mucosal barrier, a process likely inhibited by lactoferrin.⁵⁵ Lactoferrin also binds certain conserved microbial structures known as pathogen-associated molecular patterns (PAMPs), including *LPS* from the gram-negative cell wall and unmethylated *bacterial CpG* containing DNA. Lactoferrin might act as an anti-inflammatory agent by inhibiting binding of these molecules to proinflammatory receptors.⁵⁶ However, the immunoregulatory role of lactoferrin is complex because lactoferrin can also act alone or as a “partner molecule” with PAMPs to promote activation of pattern recognition receptors on immune cells.⁵⁶ Lactoferrin binding to *LPS* can also cause gram-negative bacterial permeabilization.⁵⁶ Lactoferrin inhibits entry of RNA and DNA viruses into host cells by binding host viral receptors or the viruses themselves.⁵⁷ Lactoferrin levels might be reduced in patients with CRS, especially in those patients with bacterial biofilms.⁵⁸

Collectin (collagen-lectin) proteins, such as surfactant proteins (SP-A and SP-D), C-reactive protein, and mannose-binding lectin, interact with numerous airway bacteria and can activate complement and exhibit antimicrobial properties.⁵⁹ Collectins recognize and bind to PAMPs, including *LPS*, through their calcium-dependent carbohydrate-binding domains, promoting bacterial clearance.⁵⁹

LL-37 is produced by the human nasal mucosa^{60,61} by kallikrein and other proteases from a precursor molecule, cathelicidin. LL-37 activation is regulated by serine peptidase inhibitor Kazal type 5 (SPINK5) and other protease inhibitors expressed in the epithelium.⁶² LL-37 has broad antibacterial properties and might even have effects against *Pseudomonas* species biofilms in animal models of CRS.⁶³ LL-37 might be anti-inflammatory by neutralizing *LPS*.⁶⁴ Of note, the transcription of the LL-37 gene is induced by binding of the bioactive form of vitamin D to its receptor.⁶⁵

Sinonasal epithelial cells express the 1- α -hydroxylase enzyme, which is important for synthesis of bioactive vitamin D; when sinonasal epithelial cells are exposed to inactive vitamin D precursors, they synthesize bioactive vitamin D and increase LL-37 production.⁶⁶ Thus vitamin D might contribute to airway innate immunity,⁶⁷ including in patients with CRS and allergic rhinitis.^{68,69}

Members of the α - and β -defensin families are also expressed in the sinonasal epithelium.^{70,71} Defensins are upregulated in response to bacterial or viral challenge.^{72,73} Defensins have broad

antimicrobial effects against both bacteria and fungi, likely through formation of pores in bacterial and fungal membranes. β -Defensins 2 and 3 have been shown to directly bind viruses to inhibit their entry into host cells and to activate cytokine production to alert immune cells.^{74,75} Notably, the function of cationic defensins is inhibited under high ionic strength conditions,⁷⁶ suggesting that abnormalities in ion transport, as in patients with CF, might reduce defensin function through alteration of ASL electrolyte concentrations.⁷⁷

Other antimicrobial peptides can have important roles in patients with CRSwNP. Members of the palate, lung, and nasal epithelial clone (PLUNC) family, including SPLUNC-1, have antimicrobial and surfactant properties, but levels were decreased in nasal polyps compared with those in healthy sinonasal tissue.⁵⁴ In addition to having antimicrobial properties, SPLUNC-1 affects ASL volume by inhibiting activation of the epithelial sodium channel.⁷⁸ The epithelial sodium channel mediates sodium and fluid absorption in airway epithelia. Thus reductions of SPLUNC-1 levels could have detrimental effects on MCC. Additionally, levels of epithelial defense proteins S100A7 (psoriasin) and S100A8/9 (calprotectin) are reduced in patients with CRSwNP.⁷⁹ Although antimicrobial protein levels differ between healthy and diseased sinonasal tissue, levels can vary by location within the sinonasal cavity.⁸⁰ For example, lactoferrin levels are higher in healthy UT compared with those in healthy inferior turbinate, whereas S100A7 levels are higher in inferior turbinate compared with those in UT.⁸⁰ Taken together, regional variability suggests that the sinonasal cavity is not uniform but rather has complex and unique roles dependent on specific anatomic location.

The lipids cholesteryl linoleate and cholesteryl arachidonate can contribute to the antimicrobial properties of nasal secretions,⁸¹ and their levels can be increased in nasal secretions of patients with CRS.⁸² The sinonasal mucosa also generates *reactive oxygen species*, which can directly damage bacteria. Lactoperoxidase⁸³ catalyzes the oxidation of substrates by hydrogen peroxide (H_2O_2). Airway epithelial ciliated cells produce H_2O_2 through the action of *NADPH oxidase* isoforms, including DUOX1 and DUOX2.⁸⁴ A potentially important substrate for the lactoperoxidase/ H_2O_2 system is thiocyanate. Thiocyanate is oxidized through lactoperoxidase to hypothiocyanite, a compound with antibacterial, antifungal, and antiviral effects.⁸⁵ Both CFTR and pendrin can regulate thiocyanate transport, linking ion transport with host defense.⁸⁶ CFTR defects that reduce thiocyanate secretions might impair airway innate defense mechanisms in patients with CF.⁸⁷ An increase in pendrin levels in patients with CRS can also influence thiocyanate transport.²⁹

Generation of nitric oxide (NO) by the sinonasal epithelium is thought to be critical for airway innate immunity, with the major source being the paranasal sinuses.⁸⁸ NO is generated by nitric oxide synthase (NOS). NOS isoforms vary in their mRNA inducibility, as well their sensitivity to intracellular calcium. NOS isoforms are expressed in the cilia and microvilli of epithelial cells,⁸⁹ with the maxillary sinus being a site of high expression.⁹⁰ NO activates guanylyl cyclase to increase ciliary beating through cyclic GMP and protein kinase G. NO and reactive derivatives, such as peroxynitrite,⁹¹ also directly damage bacterial proteins and DNA^{92,93} and inhibit viral replication.⁷⁵ Although studies have linked increased NO levels with host defense *in vivo*, others have suggested increased NO levels are detrimental.⁹⁴ The wide range of NO measurement methods and heterogeneous study populations have limited the conclusions that can be drawn from studies investigating NO as

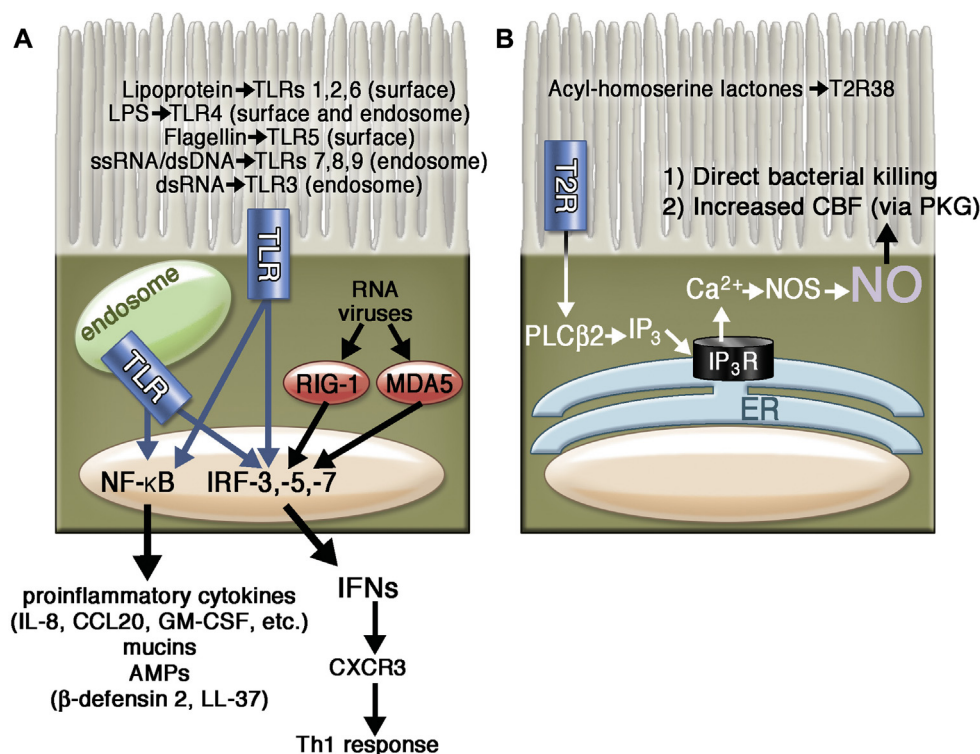


FIG 2. Role of Toll-like receptors (TLRs) and other pattern recognition receptors, as well as T2Rs, in regulation of sinonasal innate immunity by ciliated epithelial cells. **A**, TLRs located both on the cell surface and in intracellular endosomes of epithelial cells recognize PAMPs and stimulate innate immune responses. PAMPs recognized by specific TLRs are indicated in the figure. TLRs activate downstream intracellular signaling proteins myeloid differentiation primary response gene-88 (MyD88), Toll-interleukin 1 receptor domain containing adapter protein (TIRAP), translocation-associated membrane protein 1 (TRAM), and Toll-interleukin 1 receptor domain-containing adapter inducing interferon beta (TRIF) (not shown), which activate transcription factors, such as cAMP response element binding protein (CREB) (not shown), nuclear factor κB (NF-κB), and interferon response factors that activate transcription of antimicrobial peptides (AMPs), cytokines, and chemokines. The secretion of proinflammatory cytokines and interferons links innate and adaptive immunity. Additionally, the cytoplasmic helicases retinoic acid-inducible gene 1 (RIG-1) and melanoma differentiation-associated protein 5 (MDA5) recognize RNA viruses by detecting intracellular viral double-stranded RNA (dsRNA), including viral genomic RNA (vRNA). **B**, T2R38 expressed in ciliated epithelial cells recognizes bacterial homoserine lactones to stimulate calcium-dependent NOS activation and NO production. This NO diffuses into the ASL and has direct antibacterial effects. NO also acts as an intracellular signaling molecule to stimulate ciliary beat frequency through protein kinase G (PKG). ER, Endoplasmic reticulum; IP₃, inositol trisphosphate; IP₃R, inositol trisphosphate receptor; IRF, interferon regulatory factor; PLCβ2, phospholipase C isoform β2.

a diagnostic, prognostic, or efficacy indicator in patients with sinonasal disease.⁹⁴

REGULATION OF ANTIMICROBIAL COMPOUND PRODUCTION AND SECRETION

The most well-studied pathway for regulation of antimicrobial compounds by sinonasal epithelial cells is through *Toll-like receptors* (TLRs), which recognize microbial PAMPs (Fig 2, A).⁴⁵ Sinonasal cells express approximately 10 TLRs, with expression changes observed in patients with CRS.⁹⁵ TLRs stimulate the direct transcription, translation, or both of mucins and AMPs.^{45,96} TLR responses occur over the course of hours and are likely critically important during times of sustained colonization or infection. TLRs activate airway epithelial cells to secrete defense molecules, as well as cytokines and chemokines that recruit dedicated immune cells and activate inflammation, which might play an important role in CRS pathogenesis. Epithelial cells from patients with CRS produce

less IL-8 in response to TLR2 ligands, which might impair immunity. TLRs also play an important role in detection of rhinoviruses, respiratory syncytial virus, and influenza and can stimulate induction of *type I interferons*.⁷⁵

Human ciliated epithelial cells also express taste family type 2 receptor (T2R) bitter taste receptors, which were originally identified on the tongue. At least one T2R, T2R38, is expressed in ciliated epithelial cells, detects bitter acyl homoserine lactone quorum-sensing molecules secreted by gram-negative bacteria, and stimulates an increase in MCC and production of bactericidal levels of NO (Fig 2, B).²⁵ T2R38 function has also been linked to antibacterial immune responses *in vitro*,²⁵ as well as gram-negative upper respiratory tract infection²⁵ and chronic rhinosinusitis susceptibility *in vivo*.⁹⁷⁻¹⁰¹ Bitter taste receptors are also expressed in specialized epithelial cells termed solitary chemosensory cells, a specialized cell type that stimulates the rapid release of stored AMPs from surrounding cells.^{96,102} Because of the rapid innate immune responses observed during stimulation of bitter taste receptors in the airway, it appears that

they constitute an early-warning arm of the sinonasal innate immune system. T2R solitary chemosensory cells are also regulated by T1R sweet taste receptors,^{103,104} a mechanism that might be important in diabetics and patients with CRS, both of whom have increased glucose levels in their ASL.¹⁰⁵⁻¹⁰⁷ Taste receptors are emerging as a front-line defense mechanism against microbial invaders, and there are exceedingly common functional polymorphisms found in all the taste receptor genes.¹⁰⁸ Thus each subject's "bitterome," or collection of T2R polymorphisms, might define the permitted and excluded microbes, thus shaping each person's baseline microbiota.

EPITHELIAL CELL BARRIER IN HOST DEFENSE

In addition to secreting antimicrobial products, epithelial cells lining the sinonasal mucosa are involved in other aspects of host defense. Epithelial cells form a physical barrier involving *tight junctions*, *adherens junctions*, and *desmosomes* that protect the underlying sinonasal tissue from damage caused by inhaled pathogens, allergens, and other irritants. However, in patients with CRS, studies have suggested that this barrier is compromised. Reductions in levels of tight junction proteins, occludin-1, and zonula occludens 1, were observed in patients with CRSwNP compared with healthy control subjects, and this was associated with decreased epithelial electrical resistance.¹⁰⁹ Other markers of epithelial cell dysfunction commonly observed in patients with CRS include abnormal ion transport, as mentioned previously; goblet cell hyperplasia; basal cell proliferation; *acanthosis*; and *acantholysis*.

There remains some debate as to whether epithelial cells in patients with CRS are inherently dysfunctional or whether exposures to external or internal factors induce this dysregulation. In support of the former argument, *ex vivo* cultures of epithelial cells taken from patients with CRSwNP showed reduced electrical resistance when compared with healthy sinonasal tissue.¹⁰⁹ This is in contrast to another recent study that found no difference in electrical resistance in epithelial cells cultured from patients with CRSwNP, patients with CRSsNP, or healthy control subjects.¹¹⁰ Interestingly, in this latter study levels of oncostatin M, a member of the IL-6 family of cytokines, were increased in nasal polyps compared with those seen in healthy control subjects and could induce tissue permeability, disrupt tight junctions, and decrease electrical resistance in cultured human epithelial cells.¹¹⁰ It will be important to resolve whether there are epithelial cell-intrinsic defects in barrier and whether increased levels of oncostatin M and other inducers of the epithelial mesenchymal transition in patients with CRSwNP contribute to epithelial barrier dysfunction. It should be noted that loss of epithelial barrier has been reported in patients with asthma and atopic dermatitis as well, and both epithelial cell-intrinsic and extrinsic mechanisms have been discovered (eg, *filaggrin* mutations and type 2 cytokine-mediated changes).

Finally, pathogens are another possible external factor that could directly affect the sinonasal epithelial cell barrier. *P aeruginosa* was shown to transiently disrupt the tight junction proteins occludin and claudin-1 in cultured human nasal epithelial cells.¹¹¹ Additionally, *S aureus*, as well as various fungi, have been found to secrete products that could also disrupt zona occludens-1 in human nasal epithelial cells *in vitro*.¹¹² In some cases the microbes produce proteases that can either cleave the junctional proteins or activate epithelial changes through

protease-activated receptors, such as PAR2.^{113,114} It is possible that the pathogen-induced disruption of the sinonasal epithelium could promote further bacterial infection, colonization, or even biofilm formation and in turn further potentiate the overall chronic disease process.

THE HOST IMMUNE RESPONSE: INFLAMMATORY MEDIATORS

The host immune system is also thought to play a prominent role in CRS pathogenesis. The effector functions of innate and adaptive immune cells, as well as the various mediators they produce, can all contribute to the chronic inflammatory environmental characteristics of CRS (Fig 3). To this end, the type of inflammatory cytokines and chemokines identified within the sinonasal mucosa has historically been used to help define particular subsets of CRS. For example, CF has classically been characterized by a *type 1 inflammatory response*, with increased levels of IFN- γ in the sinonasal tissue compared with those in healthy control subjects.^{115,116}

In contrast, CRSwNP, one of the most extensively studied CRS subtypes, is typically regarded as having a type 2 inflammatory environment, at least in the United States and Europe (see below). Type 2 inflammation refers to a response typically driven by the type 2 cytokines IL-4, IL-5, and IL-13. Such inflammation typically is characterized by infiltration of large numbers of eosinophils, basophils, and mast cells. Thymic stromal lymphopoietin, an epithelial cell-derived cytokine that plays an important role in promoting type 2 responses, shows increased levels and enhanced activity in nasal polyps of patients with CRSwNP when compared with healthy sinonasal tissue.¹¹⁷ There remains some debate as to whether levels of IL-33 and IL-25, other epithelium-derived cytokines that promote type 2 inflammation, are increased in nasal polyps.¹¹⁸⁻¹²¹ Other classic type 2 inflammatory mediators, including IL-5, IL-13, eotaxin-1 (CCL11), eotaxin-2 (CCL24), and eotaxin-3 (CCL26), are often products of the epithelium, and their levels have been reported to be increased in nasal polyps compared with those in healthy control tissues.¹²²⁻¹²⁷

However, it is important to note that most early studies examining CRSwNP evaluated patients of European descent. More recently, studies have reported that nasal polyps from Asian patients living in Asia or second-generation Asians residing in the United States have an enhanced type 1 inflammatory environment with increased levels of IFN- γ and reduced levels of IL-5.^{128,129} It remains unclear why Asian patients are more likely to have a type 1 inflammatory profile in nasal polyps compared with patients from Western countries, but a yet-to-be-identified genetic factor might play a role.¹²⁹

Finally, unique characteristics that distinguish CRSsNP from other CRS subsets remain under debate. Historically, CRSsNP was thought to have a predominant type 1 inflammatory environment with more IFN- γ and less IL-5 expression than seen in nasal polyps from patients with CRSwNP.^{124,125} More recently, however, this classification has been under review, with several studies reporting similar levels of IFN- γ expression in nasal polyps of patients with CRSwNP when compared with UT from patients with CRSsNP, control UT, or control inferior turbinate.^{117,128} In a more comprehensive analysis, no differences in gene expression levels of several interferon family members (IFN- γ , IFN- α 2, IFN- α 8, and IFN- β 1) were found between nasal polyps from patients with CRSwNP, UT from patients with

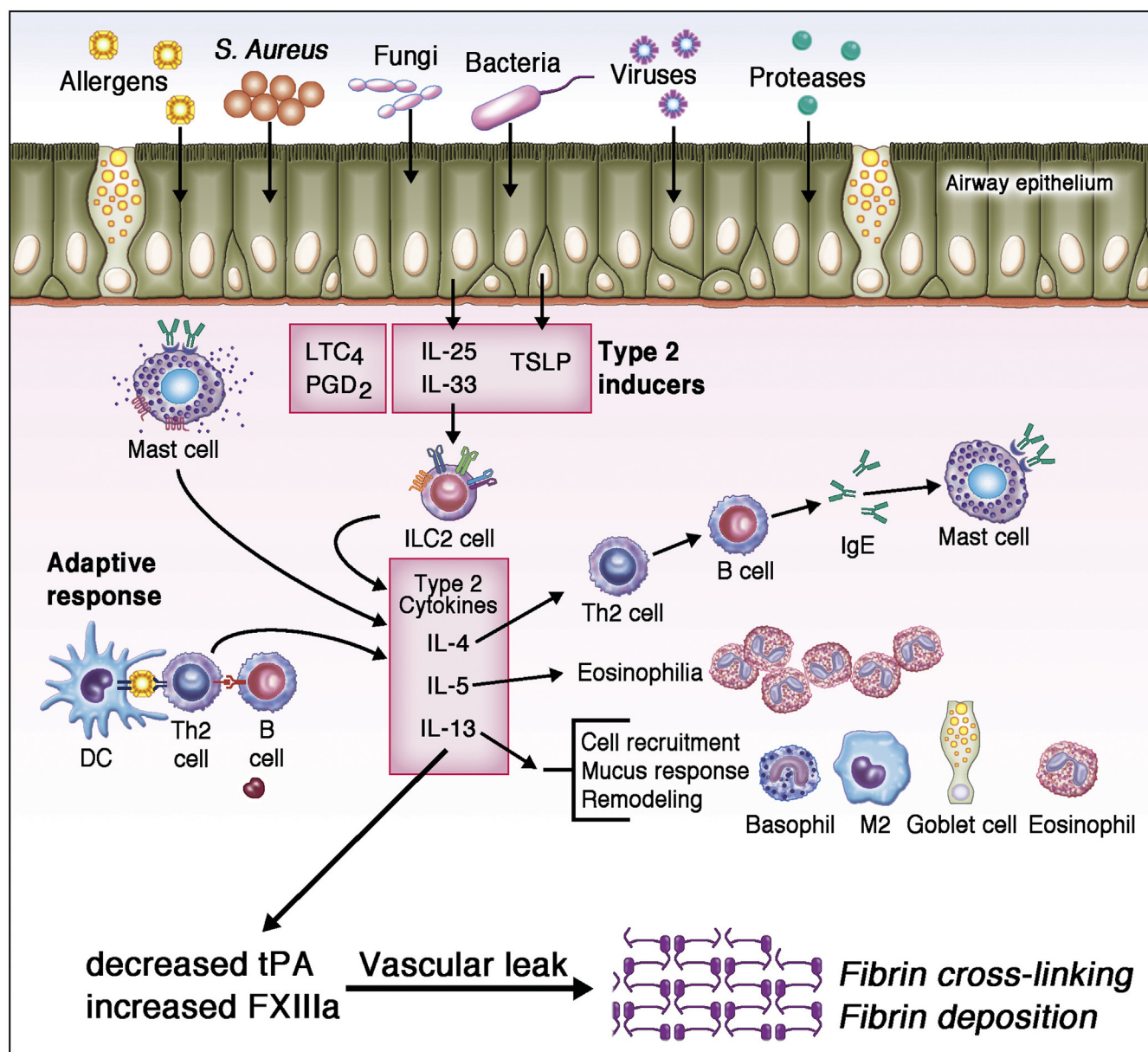


FIG 3. Role of the host immune response in patients with CRSwNP. The dysregulated epithelial barrier in patients with CRS can lead to enhanced exposures to various inhaled allergens, bacteria, fungi, and viruses. Additionally, colonization with *Staphylococcus aureus* can also occur. In nasal polyps from patients with CRSwNP, epithelial cells can release various inflammatory mediators, most notably thymic stromal lymphopoietin (TSLP), which in turn promote the development of a type 2 immune response. Numbers of innate immune cells, including innate type 2 lymphoid cells (ILC2), mast cells, and eosinophils, are all increased in nasal polyps. These cells can release type 2 cytokines that further perpetuate the ongoing inflammatory response, as well as specific granule proteins that can contribute to tissue injury. Numbers of adaptive immune cells, including both naive B cells and activated plasma cells, are also increased in nasal polyps and contribute to increased local production of antibodies within the sinonasal tissue. Finally, type 2 cytokines are also thought to contribute to decreased tissue plasminogen activator (tPA) and increased FXIIIa levels, which, in the setting of increased vascular leak, lead to increased fibrin deposition and cross-linking within nasal polyps. DC, Dendritic cells.

CRSsNP, and control UT.¹²⁶ Furthermore, no significant difference in IFN- γ protein levels was observed among all the sinonasal tissues examined.¹²⁶ Taken together, these findings suggest CRSsNP might not necessarily be more type 1 than CRSwNP. However, regional variations in protein expression

levels within the sinonasal cavity could possibly explain the discrepancies in IFN- γ expression observed among nasal polyp, ethmoid mucosa, inferior turbinate, middle turbinate, and uncinate process sinonasal tissues.⁸⁰ Given these findings, further work is needed to more extensively characterize the cytokine

environment in patients with CRSsNP, as well as in patients with CRSwNP.

THE INNATE IMMUNE RESPONSE

There have been numerous studies examining the role of innate immune cells in the development of CRS pathology. In patients with CRSwNP, **type 2 innate lymphoid cells** are thought to be early contributors to the **type 2 inflammatory response**. Numbers of these specialized innate effector cells are increased in nasal polyps and are activated by epithelium-derived cytokines, such as thymic stromal lymphopoietin and IL-33, to secrete IL-5 and IL-13.^{130,131} IL-5 is a potent activator and survival factor for eosinophils, and studies have shown that type 2 innate lymphoid cell numbers are doubled in eosinophilic compared with noneosinophilic nasal polyps.¹³²

While eosinophils are one of the major hallmarks of Western nasal polyps, mast cells and basophils, other type-2 innate inflammatory cells, are also elevated in CRSwNP compared to healthy controls.^{133,134} These innate effector cells can release a variety of inflammatory mediators and toxic granules that can perpetuate the chronic type 2 inflammatory response and induce sinonasal mucosal damage. Interestingly, a unique subset of mast cells was found in nasal polyp glandular epithelial cells that produced tryptase, carboxypeptidase A3, and chymase.¹³⁴ Because chymase is a known inducer of mucus, it is hypothesized that these specialized mast cells might play a role in the overproduction of mucus commonly seen in patients with CRSwNP.¹³⁴ Furthermore, a recent small study in patients with CRSwNP reported that mast cells might be a reservoir for *S aureus* and thus contribute to the chronicity of this infection in certain patients.¹³⁵

In contrast to nasal polyps from Western patients, nasal polyps from Asian patients are characterized by reduced numbers of eosinophils, as well as decreased levels of IL-5, eotaxin, and eosinophil cationic protein, a protein found in eosinophil granules.^{128,129} In patients with CF, neutrophils and macrophages are commonly detected.^{115,116} Unfortunately, no single defining innate effector cell has been identified in patients with CRSsNP to date, but this CRS subtype is associated with a lack of type 2 inflammation, as previously discussed.

THE ADAPTIVE IMMUNE RESPONSE

Along with the innate immune response, the adaptive immune system also contributes to the chronic inflammation seen in patients with CRS. T cells represent a major component of adaptive immunity and, despite conflicting reports in patients with CRSsNP, CD3⁺ T-cell counts have been shown to be increased in nasal polyps compared with those in healthy sinonasal tissue.^{124,127} On further analysis of CD3⁺ T-cell populations by using flow cytometry, no differences were reported in numbers of either **CD4⁺ T cells** or **CD8⁺ T cells** in nasal polyps of patients with CRSwNP versus inferior turbinates of patients with CRSsNP.¹²⁷ However, in this study the ratio of CD4⁺ to CD8⁺ T cells was significantly higher in patients with CRSwNP than in those with CRSsNP.¹²⁷ Finally, there have been several conflicting reports regarding the importance of **regulatory T (Treg) cells** in CRS pathogenesis. Van Bruaene et al¹²⁵ initially identified a decrease in forkhead box protein 3 expression, as well as expression of the regulatory cytokine TGF- β . A later study found that suppressor of cytokine signaling 3 could negatively regulate forkhead box protein 3

expression in human airway mucosa and that suppressor of cytokine signaling 3 protein levels were increased in inflammatory cells in the airway mucosa.¹³⁶ These findings suggested that Treg cells were impaired in patients with CRSwNP, thus leading to an imbalance in proinflammatory and anti-inflammatory responses. However, a later study using flow cytometric analyses reported increased numbers of Treg cells in patients with CRSwNP.¹³⁷ Taken together, further studies examining Treg cells are needed to elucidate their role in CRSwNP and to determine whether they are important in CRSsNP pathology.

In addition to T cells, B cells can also contribute significantly to the ongoing sinonasal inflammation observed in patients with CRS.¹³⁸ In patients with CRSwNP, numbers of naive B cells, as well as activated plasma cells, were found to be increased in nasal polyps when compared with those in tissue from patients with CRSsNP or healthy control tissue.¹³⁹⁻¹⁴¹ This influx in B cells might be secondary to the increased levels of the B-cell chemotactic factors CXCL13 (B cell-attracting chemokine 1) and CXCL12 (stromal cell-derived factor 1) observed in nasal polyps.¹⁴² Levels of B cell-activating factor of the TNF family and IL-6, both mediators important in B-cell activation and proliferation, are increased in nasal polyps compared with those in control subjects, with levels of B cell-activating factor of the TNF family strongly correlating with expression of CD20, another B-cell marker.^{140,143}

Interestingly, B cells in nasal polyps also appear to have local effector functions. Levels of IgG₁, IgG₂, IgG₄, IgA, IgE, and IgM were all increased in nasal polyps versus those in healthy sinonasal tissue.¹⁴¹ Importantly, there was no concomitant increase in levels of these antibodies in the peripheral blood of the same patients with CRSwNP, suggesting that B-cell antibody production in patients with CRSwNP is not a systemic response but rather driven by a stimulus within the local sinonasal inflammatory environment.¹⁴¹

Unfortunately, the specificity of the majority of antibodies detected locally in nasal polyps of patients with CRSwNP remains unclear. A certain subset of patients with CRSwNP who are also colonized with *S aureus* can have specific IgE antibodies directed against *S aureus* enterotoxins within nasal polyp tissue.¹⁴⁴ Additionally, other studies have reported increased levels of IgG and IgA autoantibodies, particularly against nuclear antigens, which are locally produced in nasal polyps.¹⁴⁵ However, it remains unclear how these autoantibodies might contribute to disease pathology in patients with CRSwNP.

Finally, even less is known regarding the role that B cells might play, if any, in CRSsNP. Numbers of naive B cells and plasma cells were not increased in UT from patients with CRSsNP compared with those in control subjects.¹⁴¹ Additionally, there were no significant increases in levels of the immunoglobulin subtypes IgG, IgA, IgE, or IgM¹⁴¹; IgA or IgG autoantibodies¹⁴⁵; or specific IgE to *S aureus*¹⁴⁴ locally in sinonasal tissue from patients with CRSsNP versus that from healthy control subjects. Interestingly, however, patients with CRSsNP are unique in that IgD levels are locally increased within the sinonasal tissue in this population, unlike in healthy control subjects or patients with CRSwNP.¹⁴⁶

TISSUE REMODELING

As in those with other chronic inflammatory diseases, tissue remodeling also occurs in patients with CRS. However, the histologic characteristics, as well as mechanisms contributing to

this upper airway remodeling, are hypothesized to differ between patients with CRSsNP and those with CRSwNP. Traditionally, CRSsNP is characterized by fibrosis, basement membrane thickening, and goblet cell hyperplasia. Levels of TGF- β , a key mediator in promoting fibrosis and airway remodeling, were found to be increased in patients with CRSsNP compared with those in patients with CRSwNP and healthy control subjects.¹⁴⁷ Likewise, expression of the TGF- β receptor, as well as mediators critical in TGF- β signaling, was also enhanced in sinonasal tissue of patients with CRSsNP.¹⁴⁷ Interestingly, there appears to be some regional variation in TGF- β protein expression throughout the sinonasal cavity, with the inferior and middle turbinates having the lowest level of expression in patients with CRSsNP.¹⁴⁸

In contrast to CRSsNP, CRSwNP is histologically characterized by a significant inflammatory cell infiltrate, formation of pseudocysts, and stromal tissue edema. Collagen levels are reduced in nasal polyps,¹⁴⁹ but there have been conflicting reports regarding TGF- β . One study suggests that TGF- β levels are reduced in patients with CRSwNP,¹²⁵ whereas another study reported increased levels when compared with those in healthy control subjects, patients with CRSsNP, or both.¹⁵⁰ More recently, TGF- β expression was found to be less in epithelial cells but increased in stromal cells in nasal polyps compared with the same cell subtypes in healthy sinonasal tissue.¹⁵¹ These regional differences might also explain recent conflicting reports regarding the expression levels of Activin A, a member of the TGF- β superfamily that is hypothesized to play a role in remodeling in nasal polyps.^{152,153}

Another aspect of CRSwNP pathogenesis relates to the growth of nasal polyps themselves. In general, plasma proteins are enriched in affected sinus and polyp tissue because of vascular leak and can traverse the dysfunctional epithelial barrier into the lumen in patients with CRS. In nasal polyps studies have shown that *fibrin* deposition is increased, which in turn can form a scaffold, trapping plasma proteins and enhancing tissue edema.¹⁵⁴ This fibrin mesh is further stabilized by Factor XIIIa, levels of which are also increased in patients with CRSwNP and thought to be another signature of a type 2 inflammatory environment.¹⁵⁵ Additionally, nasal polyps also have reduced levels of *tissue plasminogen activator*, an enzyme critical for the breakdown of fibrin mesh, as well as *D-dimer*, a product of fibrin degradation.¹⁵⁴ Taken together, these studies suggest that an imbalance in fibrin formation (increased fibrin and *Factor XIIIa* levels) and degradation (reduced tissue plasminogen activator and D-dimer levels) might contribute to the polyp growth observed in patients with CRSwNP (Fig 3).

CONCLUSIONS

Significant advances have been made in the understanding of CRS pathogenesis. Mechanisms involving MCC, epithelial barrier dysfunction, the host immune response, and tissue remodeling all are thought to work in concert and contribute to the chronic inflammation characteristic of patients with CRS. It is the goal of all working in this field that laboratory and clinical findings will continue to build the foundation on which more developmental studies can be performed that advance diagnostic and therapeutic strategies for the benefit of affected patients.

Fig 3 is a composite of 2 previous figures prepared by Ms Jacqueline Schaffer, and we would like to acknowledge her contribution.

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