

Mechanisms and pathogenesis of chronic rhinosinusitis



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Chronic rhinosinusitis (CRS) is a heterogeneous disease characterized by local inflammation of the upper airways and is historically divided into 2 main phenotypes: CRS with nasal polyps and CRS without nasal polyps. Inflammation in CRS is mainly characterized by 3 endotypes based on elevation of canonical lymphocyte cytokines: type (T) 1 (T_H1) by T_H1 cytokine IFN- γ , T₂ by T_H2 cytokines IL-4, IL-5, and IL-13, and T₃ by T_H17 cytokines including IL-17. Inflammation in both CRS without nasal polyps and CRS with nasal polyps is highly heterogeneous, and the frequency of various endotypes varies geographically around the world. This finding complicates establishment of a unified understanding of the mechanisms of pathogenesis in CRS. Sinonasal epithelium acts as a passive barrier, and epithelial barrier dysfunction is a common feature in CRS induced by endotype-specific cytokines directly and indirectly. The sinonasal epithelium also participates in both innate immunity via recognition by innate pattern-recognition receptors and promotes and regulates adaptive immunity via release of chemokines and innate cytokines including thymic stromal lymphopoietin. The purpose of this review was to discuss the contribution of the epithelium to CRS pathogenesis and to update the field regarding endotypic heterogeneity and

various mechanisms for understanding pathogenesis in CRS. (*J Allergy Clin Immunol* 2022;149:1491-503.)

Key words: Chronic rhinosinusitis, endotype, epithelial dysfunction, eosinophils, nasal polyps, neutrophils

Chronic rhinosinusitis (CRS) is one of the most common chronic inflammatory diseases and affects approximately 10% of the population worldwide. CRS is a heterogeneous disease characterized by local inflammation of the upper airways and sinuses that persists for at least 12 weeks and induces a significant diminution in quality of life.^{1,2} Although CRS is a broad syndrome characterized by many features in individuals such as presence of asthma comorbidity, aspirin sensitivity, allergic fungal sinusitis, and cystic fibrosis in addition to rhinosinusitis, it is historically divided into 2 main phenotypes on the basis of presence or absence of nasal polyps (NPs): CRS with NPs (CRSwNP) and CRS without NPs (CRSsNP). Initial studies suggested that these 2 phenotypes were considered to be characterized by distinct endotypes by the classical T_H1/T_H2 theory²⁻⁶; however, recent studies have revealed that inflammation in both CRSsNP and CRSwNP is highly heterogeneous and each phenotype can manifest the 3 main inflammatory endotypes: type (T) 1, T₂, and T₃ based on the elevation in canonical T-cell cytokines (T_H1, T_H2, and T_H17, respectively). It is also known that the frequency of various endotypes varies geographically around the world and even within a single country (see below).⁷⁻⁹ This finding complicates establishment of a unified understanding of the mechanisms of pathogenesis in CRS. The importance that sinonasal epithelial cell function has in the pathogenesis of CRS is now clearly established. This review will update the current knowledge of the pathogenic mechanisms of CRS by focusing the discussion on epithelial contributions and endotypic-specific mechanisms.

EPITHELIAL CONTRIBUTIONS TO CRS

The past several decades have seen a gradual but definitive shift in our view of the sinonasal epithelium from a passive barrier to an active immunologic organ with both innate and adaptive components (Fig 1). In parallel, multiple investigations have demonstrated that the inflammatory profiles associated with CRS are often predicated upon the dysfunctional regulation of these complex mechanisms with both downstream and reciprocal sequelae.

Passive structural epithelial contributions to CRS

The sinonasal mucosa is one of the initial sites of interaction between the body and extrinsic inhaled pathogens. Consequently,

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Abbreviations used

AERD:	Aspirin-exacerbated respiratory disease
AMP:	Antimicrobial peptide
CLC:	Charcot-Leyden crystal
CRS:	Chronic rhinosinusitis
CRSsNP:	CRS without NPs
CRSwNP:	CRS with NPs
DC:	Dendritic cell
EMT:	Epithelial-to-mesenchymal transition
EPI:	Endogenous protease inhibitor
ILC2:	Group 2 innate lymphoid cell
LT:	Leukotriene
MCC:	Mucociliary clearance
MC _T :	Mast cell-tryptase
MCTC:	Mast cell-tryptase/chymase
NP:	Nasal polyp
OSM:	Oncostatin M
PG:	Prostaglandin
P-gp:	P-glycoprotein
SCFA:	Short-chain fatty acid
STAT6:	Signal transducer and activator of transcription 6
T:	Type
TLR:	Toll-like receptor
tPA:	Tissue plasminogen activator
TSLP:	Thymic stromal lymphopoietin

structural competence of the epithelium represents the most fundamental mechanical contribution to innate immunity. When barrier disruption occurs, enhanced exposure to foreign activators and antigens results in an active immune response.¹⁰ Multiple studies have demonstrated evidence for barrier dysfunction in the setting of CRS characterized by both a reduction in tight junctions and increased ion permeability.^{10,11} For example, decreased expression of the tight junction proteins occludin-1 and zonula occludens 1 was demonstrated in CRSwNP relative to healthy controls.¹² Similarly, alterations in E-cadherin,¹³ shortening of desmosomes,¹⁴ and reductions in claudin-1¹⁵ have all been reported in CRS mucosal samples. Several lines of evidence have suggested that interactions with external pathogens may initiate these changes. *Pseudomonas aeruginosa* has been shown to disrupt both occludin and claudin-1,¹⁶ whereas *Staphylococcus aureus* has been found to secrete products disruptive to human zona occludens-1.¹⁷ In T2 conditions, IL-4 and IL-13 are known to reduce epithelial barrier function in sinonasal epithelial cells.¹² Activation of eosinophils also plays a role in barrier dysfunction through release of granule proteins and eosinophil extracellular traps.^{18,19} In non-T2 conditions, the T1 cytokine IFN- γ reduces epithelial barrier function in sinonasal epithelial cells.¹² Other inflammatory mediators such as oncostatin M (OSM), a member of the IL-6 family, released from neutrophils have been observed to be elevated in CRSwNP, resulting in increased tissue permeability secondary to both tight junction and bioelectric disruption.^{20,21} Granzymes produced from natural killer cells may also contribute to barrier dysfunction.²² Although the kinetics of barrier dysfunction in CRS continues to be studied, similar defects have been observed in both asthma and atopic dermatitis in which both intrinsic and extrinsic mechanisms have been implicated.²³

Active structural epithelial contributions to CRS

The active structural counterpart to the airway mucosal barrier is mucociliary clearance (MCC), which functions to clear the mucosal surface of particulates and irritants. MCC is regulated by small molecule neurotransmitters and neuropeptides that influence both mucus layer secretion²⁴ and ciliary beat frequency.²⁵ The importance of MCC to proper immune function is highlighted by disorders such as cystic fibrosis and primary ciliary dyskinesia, in which impaired MCC contributes to chronic sinonasal inflammation and infection.^{26,27} In the setting of CRS, acquired ciliary dysfunction with a blunting of beat response to promotility stimuli following exposure to microbial toxins and/or intrinsic inflammation is evident.^{28,29} Indeed, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *S aureus*, *Aspergillus fumigatus*, and *P aeruginosa* have all been shown to produce compounds that impair MCC.²³

Functional epithelial contributions to CRS

The structural features of innate epithelial immunity are complemented by an array of functional receptors capable of recognizing pathogenic epitopes and elaborating a rapid immune response. Pattern-recognition receptors are germline-encoded receptors that recognize a wide range of conserved microbial structures known as pathogen-associated molecular patterns.³⁰ The Toll-like receptors (TLRs) are a class of pattern-recognition receptors that are T1 integral membrane glycoproteins characterized by a recognition domain containing varying numbers of leucine-rich-repeat motifs and a transmembrane signaling domain.³¹ Although some studies have reported upregulation of 1 or more TLRs,³²⁻³⁵ others have reported reduced levels or no differences.³⁶ Taste receptors have also been established as participants in innate epithelial immunity. Polymorphisms in the bitter taste receptor T2R38 have been shown to underlie the lack of ability to taste bitter compounds in food. The nonfunctional allele of T2R38 (AVI) has been established as a risk allele for disease severity in both CRSsNP and cystic fibrosis.^{37,38} The mechanism has been traced to insensitivity to acyl-homoserine lactone compounds, quorum-sensing molecules produced by gram-negative bacteria in biofilms, which normally activate T2R38-induced Ca²⁺ responses, leading to increased MCC and production of the antimicrobial gas NO.^{39,40}

TLRs have similarly been widely studied with regard to their ability to regulate rapid antimicrobial peptide (AMP) release by both epithelial and airway solitary chemosensory cells in response to PAMP recognition and taste receptor transduction, respectively.⁴¹ Some of the most widely studied AMPs include lysozyme, lactoferrin, antitrypsin, defensins, S100 proteins, and surfactants.⁴² AMPs have been shown to have significant roles in CRS. For example, epithelial defense proteins S100A7 (psoriasin) and S100A8/9 (calprotectin) are reduced in CRSwNP.⁴³ Members of the palate, lung, and nasal epithelial clone family, including SPLUNC-1, are similarly decreased in CRSwNP compared with healthy controls.⁴⁴ SPLUNC-1 also influences airway surface liquid volume by inhibiting activation of the epithelial sodium channel, which mediates sodium and fluid absorption in airway epithelia, thereby potentially impacting MCC.⁴⁵ Airway surface liquid may additionally be influenced by the apically expressed anion exchanger pendrin/SLC26A4, which has been reported as upregulated in CRSwNP, leading to reduced volumes and subsequent MCC dysfunction.⁴⁶

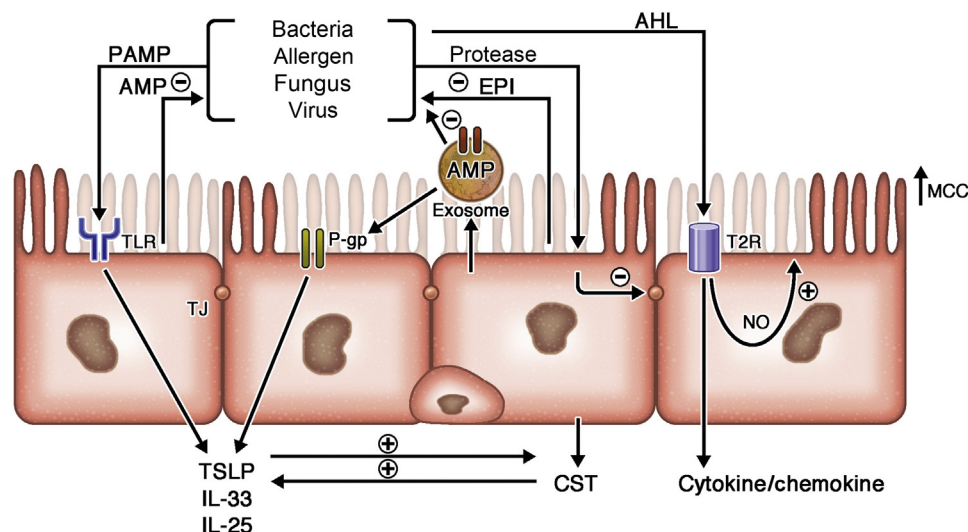


FIG 1. Epithelial contributions to CRS. Extrinsic pathogens and irritants interact with multiple epithelial receptors to induce both innate and adaptive immune responses. Protease exposure induces tight junction dysfunction and secretion of EPIs, which neutralize extrinsic proteases and stimulate type 2 inflammation. Similarly, PAMPs interact with TLRs, leading to apical AMP secretion as well as epithelial-derived cytokine secretion. P-gp functions to clear the cytoplasm of environmental toxins while reinforcing epithelial cytokine release. Epithelial-derived exosomes support these innate immune responses by shuttling AMPs directly to mucus-borne pathogens while promoting the interepithelial transfer of P-gp. Bacterial-derived quorum-sensing molecules, such as AHL compounds, also interact with bitter taste receptors such as T2R38 to induce bacteriocidal NO release, enhanced MCC, and cytokine/chemokine secretion. AHL, Acyl-homoserine lactone; CST, cystatin; NO, nitric oxide; PAMP, pathogen-associated molecular pattern.

Although AMPs may be directly released from epithelial cells, they have also been found within nasal mucosa-derived exosomes.⁴⁷ Exosomes are 30- to 150-nm, tetraspanin-enriched microvesicles, which are secreted into almost all body fluids, including blood, lymph, and nasal mucus. Nocera et al⁴⁷ demonstrated that TLR4 stimulation of the nasal epithelium resulted in 2-fold increase in nasal mucosa-derived exosome release containing both inducible NO synthase and multiple AMPs, providing significant microbiocidal activity against *Paeruginosa*.

Although pattern-recognition receptors play a critical role in the innate immune response through direct antipathogen activity, they also promote the secretion of an array of proinflammatory cytokines and chemokines that function to recruit immune cells such as T cells, B cells, basophils, and eosinophils into the local epithelial microenvironment. These epithelial-derived cytokines including thymic stromal lymphopoietin (TSLP), IL-25, IL-33, and B-cell-activation factor of the TNF family have all been associated with enhancement of adaptive immunity and CRS-associated airway inflammatory responses.³¹ One of the epithelial signatures of inflammation is overexpression of P-glycoprotein (P-gp) within the cell membrane.⁴⁸ P-gp is a 170-kDa glycoprotein encoded by the *MDR1* (ABCB1) gene located on chromosome 7q21.12. P-gp is regionally upregulated in CRS, may be transported between epithelial cells through exosome shuttling,⁴⁹ and has a spectrum of expression that is most profound in T2 disease.^{50,51} P-gp has been shown to modulate epithelial cytokine release in a concentration-dependent manner^{52,53} and is also capable of potentiating glucocorticoid activity.^{54,55} These findings suggest that P-gp plays an important role in reinforcing epithelial-derived cytokine-mediated inflammation in CRS. This concept was supported by a recent clinical trial

demonstrating improvement in both subjective and objective measures of CRSwNP following inhibition of P-gp using verapamil HCl.⁵⁶

Epithelial protease/protease inhibitor imbalance in CRS

Both structural and functional innate epithelial immune responses evolved to rapidly respond to a near-constant exposure to extrinsic environmental pathogens, while enabling an efficient return to a healthy homeostatic state. In the setting of CRS, there is a failure to downregulate the inflammatory cascade, which has led to multiple etiopathologic hypotheses focusing on host epithelial-environmental interactions to both fungus^{57,58} and bacteria. *S aureus* “superantigens” have also been explored, suggesting a potential contributory role in some forms of CRS.⁴⁸ These lines of research suggest a common unifying pathway of excessive protease exposure. Indeed, allergens such as dust mites, fungi, and bacteria all possess significant intrinsic protease activity.⁵⁹⁻⁶¹ These exogenous proteases appear to be capable of causing both barrier dysfunction and inducing the release of epithelial-derived cytokines, which, in turn, activate TH2 cells and group 2 innate lymphoid cells (ILC2s) to secrete T2 cytokines.¹ In response to exogenous protease exposure, the epithelium is also driven to produce endogenous protease inhibitors (EPIs). In some cases, these EPIs such as cystatin A and serine protease inhibitor SPINK5 appear to be underexpressed in CRS.⁶² In other cases however, EPIs such as cystatin SN, a cysteine protease inhibitor, have been shown to be significantly increased in the epithelium from patients with eosinophilic CRS (T2 CRS) versus non-T2 CRS.^{63,64} Cystatin SN is capable

of inducing TSLP production and is reciprocally upregulated by TSLP and IL-33.⁶⁵ Cystatin SN has also been shown to induce cultured NP fibroblasts to increase periostin expression, which, in turn, is associated with recruitment and activation of eosinophils.^{66,67} Taken together, these studies suggest that a general imbalance between exogenous proteases and EPIs may function as upstream drivers of epithelial-derived inflammation in CRS.

Reciprocal epithelial responses to airway inflammation

The paradigm shift in our conception of the epithelium as an active immunomodulator in response to apical stimuli is mirrored in our evolving understanding of reciprocal epithelial interactions with basolateral inflammatory signaling. For example, airway epithelial cells are capable of inducing dendritic cell (DC) migration into the epithelium via CCL20 (MIP-3 α) in response to inhaled foreign antigens.³² CCL20 is the only chemokine known to interact with CCR6 that is expressed by immature DCs and Langerhans cells.⁶⁸ The epithelial cells subsequently regulate DC control of T_H differentiation through local production of TSLP, a DC activator that itself may be further stimulated by IL-4 exposure.⁶⁹

Another significant epithelial response to chronic local inflammation is the induction of fibrosis and tissue remodeling. Disorders of both the coagulation and fibrinolytic systems have been demonstrated in CRS in both clinical and animal models. A combination of thrombin activation, decreased t-PA activity, and increased coagulation factor XIII-A expression functions to induce enhanced fibrin deposition within NPs (see below).⁷⁰ Both thrombin and protease-activated receptor-1 agonist peptides were capable of stimulating vascular endothelial growth factor production *in vitro*. Taken together, these studies show that activation of the coagulation system, coupled with decreased fibrinolysis, contributes to significant mucosal remodeling in CRS through the trapping of plasma proteins, enhanced edema, pseudocyst formation, and vascular endothelial growth factor secretion. Thrombin may even further exacerbate the epithelial inflammatory response through the stimulation of IL-6, IL-8, prostaglandin (PG)E₂, CCL2, platelet-derived growth factor, and MUC5AC from airway epithelial cells.⁷¹⁻⁷³ These features of coagulation cascade derangement have also been postulated to explain the site specificity of NP generation. Levels of the plasminogen activators, urokinase-type plasminogen activator (uPA) and tissue plasminogen activator (tPA), appear to be lower in middle meatal mucosa relative to the inferior turbinate, suggesting that increased susceptibility to fibrin deposition may be regional and favor the middle meatus.⁷⁴

Further studies into epithelial remodeling in the setting of CRS have focused on fibrosis within the lamina reticularis as well as goblet cell proliferation. Signal transducer and activator of transcription 6 (STAT6) activation in airway epithelial cells has been shown to induce IL-13-dependent airway inflammation in a murine model.^{75,76} IL-13, along with epidermal growth factor receptor signaling, is important in both fibrogenic responses and regulation of mucus-producing cell types. Similarly, periostin may be induced by both IL-13 and IL-4, which is capable of binding the extracellular matrix proteins tenascin-C, fibronectin, and collagens, all of which are involved in the fibrosis response.⁷³ Polymorphisms in matrix metalloproteinase-9, which functions to degrade extracellular matrix proteins, have been reported as a risk gene in CRS.⁷⁷ TGF- β is an additional important mediator of airway fibrosis.

Both TGF- β and TGF- β signaling have been shown to be elevated in CRSsNP tissue relative to controls.⁷⁸ When analyzed among CRSwNP however, conflicting data have been reported, with studies showing a range of levels as compared with controls.^{79,80}

Epithelial-to-mesenchymal transition and cellular diversity in CRS

The confluence of barrier dysfunction, fibrosis, and remodeling described in CRS may be conceptualized within the broader concept of epithelial-to-mesenchymal transition (EMT). EMT has been studied in multiple T2 inflammatory diseases and has been identified within the context of CRS.^{81,82} EMT generally results from injury to the epithelium leading to disruption of homeostasis with a subsequent dynamic repair process designed to ameliorate further extrinsic damage. Indeed, environmental proteases including those from respiratory allergens such as *Dermaphagoides pteronyssinus* have been shown to initiate EMT.⁸³ Within the sinonasal epithelium, EMT is characterized by a loss of tight junctional proteins including ZO-1 and occludin. Hupin et al⁸⁴ reported decreased E-cadherin in CRS, along with decreased cytokeratins and vimentin, which correlated with basement membrane thickness and tissue eosinophilia. Junctional protein losses result in a separation from the basement membrane, loss of polarity, and increased cell division to cover the area of injury. Simultaneously, mesenchymal cells differentiate from basal epithelial cells and elaborate multiple extracellular matrix proteins such as desmin, fibronectin, tenascin, laminin, and collagens to provide a biologic dressing to the deepithelialized areas. Specific factors that have been associated with EMT and acanthosis in CRS have included TGF- α , OSM, epiregulin, and HIF1 α .^{20,21,36} Periostin is also a significant contributor to EMT via a TGF- β -dependent pathway.^{64,85}

EMT has been leveraged to interrogate the temporospatial aspects of NP formation whereby early-stage polyps have been associated with more active EMT markers including TGF- β , more vimentin staining, elevated α -smooth muscle actin-positive activated myofibroblasts, increased fibronectin, and higher numbers of M2 macrophages. Similarly, more extensive deposition of collagen was noted in polyp stalks, indicating greater fibroblast activity during the initial stages of polyp formation.⁸⁶

Recent studies into additional cellular changes within the context of CRS using massively parallel single-cell RNA sequencing have provided further insight into changes in the epithelial cellular ecosystem in the setting of CRS. These investigations identified a global reduction in cellular diversity with NPs as compared with control tissue characterized by basal cell hyperplasia, a reduction in glandular cells, and a shift in secretory cell transcriptomes including, among others, a significant increase in the expression of EPIs including cystatin SN.⁸⁷

INFLAMMATORY ENDOTYPES IN CRS

Recent studies suggest that inflammation in CRS is highly heterogeneous and can be separated by 3 main inflammatory endotypes: the T1 endotype by the elevation of T1 cytokine IFN- γ , the T2 endotype by eosinophilia and the elevation of T2 cytokines, and the T3 endotype by neutrophilia and elevation of T3 cytokines including IL-17A. A recent study elucidated associations between endotypes and clinical presentations and found some associations in that the T2 endotype is associated with smell loss,

asthma comorbidity, and nasal polyposis, whereas the T3 endotype is associated with presence of intraoperative pus, suggesting a link with infection.⁹ However, most endotype-associated studies only separated 2 main endotypes, eosinophilic (T2) and noneosinophilic (non-T2), which contains several subendotypes including T1 and T3 (neutrophilia), until recently. Indeed, earlier studies compared these 2 groups in CRS.⁸⁸⁻⁹² On the basis of this limitation, we mainly describe T2- and non-T2-mediated inflammation in CRS.

INFLAMMATION IN CRSwNP

The T2 endotype is a common endotype in CRSwNP in Western countries.^{3,7-9} In addition, the prevalence of T2 CRSwNP has been increasing over the past decades in Asia similar to other allergic diseases such as asthma and allergic rhinitis.^{3,7,93-96} There is geographic variability even within a single country, and indeed the presence of T2 CRSwNP was very different between Beijing (60%) and Chengdu (20%) in China.⁷ This implies that the inflammatory endotype is controlled by microenvironments more than genetic factors.

T2 CRSwNP

T2 cytokines including IL-4, IL-5, and IL-13 produced by T_H2 cells, ILC2s, and mast cells play important roles in T2 CRSwNP (Fig 2). IL-5 primarily is involved in eosinophilia and also participates in activation of plasma cells in NPs. IL-4 and IL-13 are key factors that control many important factors in NPs including IgE responses, epithelial barrier dysfunction, mucus production, remodeling, and fibrin deposition.³ Treatment with an anti-IL-4R α antibody (dupilumab) that inhibits both IL-4 and IL-13 signaling reduced NP size and improved quality of life and symptoms including nasal congestion and anosmia in patients with severe CRSwNP.⁹⁷ Several groups performed transcriptomic approaches on CRSwNP in Western countries (mostly T2) and eosinophilic (T2) CRSwNP in Asia, identified upregulated genes in T2 NPs, and predicted immunologic and inflammatory mechanisms in CRSwNP more globally.^{91,92,98,99} The upregulated genes in T2 NPs included cell markers and chemokines for eosinophils (eg, *CLC*, *CCR3*, *CCL13*, and *CCL26*), macrophages (especially M2 phenotype)/myeloid DCs (eg, *CCR1*, *CD163*, *F13A1*, *CCL2*, and *CCL18*), T_H2/ILC2 (eg, *IL1RL1*, *IL17RB*, and *PTGDR2*), mast cells (eg, *CPA3* and *TPSAB1*), epidermal differentiation (eg, *KRT6A* and *SPRR1B*) and extracellular matrix (eg, *FN1* and *FGF1*), and other common IL-4/IL-13-induced genes (eg, *CST1* [cystatin SN] and *POSTN* [periostin]).^{91,92,98,99} Nakayama et al⁹⁹ also directly compared NPs from Asian (residing in Japan) and White (residing in the United States) patients with CRSwNP by RNA sequencing and found that the gene expression profile in T2 NPs from both groups showed near-complete overlap. This suggests that key molecular mechanisms of T2 NPs in Asian and Western countries are probably the same. Because immune and inflammatory cells and their factors play central roles in T2 CRSwNP, we will further introduce the presence and role of each cell type in this section.

ILC2s and T_H2 cells. Antigen-independent T2 inflammation is mainly controlled by ILC2s. ILC2s are known to be highly elevated in Western T2 NPs.¹⁰⁰⁻¹⁰² In Asia, ILC2s are elevated in T2 NPs but not in non-T2 NPs.¹⁰³ Poposki et al¹⁰¹ found that fluorescence-activated cell sorting-sorted NP ILC2s but not

peripheral blood ILC2s spontaneously released IL-5 and IL-13, suggesting that ILC2s in NPs are highly activated and release T2 cytokines *in vivo*. ILC2s express receptors responsive to epithelial cell-derived innate T2 inducers, TSLP, IL-25, and IL-33. In the case of CRS, elevated TSLP in T2 NP tissue was found across the world.^{104,105} TSLP is induced by viruses, protease-containing allergens, and T2 cytokines IL-4 and IL-13 in epithelial cells, and the combination of these stimuli synergistically or additively enhanced TSLP production.^{69,104,106} TSLP activity in NP tissue was enhanced by the posttranslational modifications from tissue proteases, and these active TSLP metabolites stimulate ILC2s as well as DCs and mast cells much more potently than mature TSLP.^{104,107} Importantly, anti-TSLP (tezepelumab) has been approved for treatment with asthma and is in clinical trial for CRSwNP.¹⁰⁸ In contrast, elevation of other epithelial-derived innate T2 inducers, IL-25 and IL-33, is controversial. Elevation of IL-25 in T2 NPs was found mainly in Asia, and a US study also demonstrated that IL-25 was detected in a minor subset of epithelial cells called solitary chemosensory cells and was elevated in NPs.^{105,109} In contrast, the other US study showed almost undetectable levels of IL-25 in NPs.¹⁰⁵ This may suggest that IL-25 plays a role in a limited area of NPs or on an early event in NP pathogenesis. In the case of IL-33, mixed results were reported in T2 NPs throughout the world.¹⁰⁵ However, because IL-33 requires 2 steps to obtain the cytokine activity, release from the nucleus and cleavage by enzymes, we cannot draw conclusions based only on the expression of IL-33.^{110,111} Detailed studies examining whether IL-25 and IL-33 play a role in the pathogenesis of T2 CRSwNP are still required.

It is implicated that activation of 4 distinct transcriptional pathways (NF- κ B, nuclear factor of activated T cells [NFAT], STAT5, and STAT6) is needed for the most robust generation of T2 cytokines in ILC2s.¹¹² Published studies suggested that activators of nuclear factor kappa B (receptor activator of nuclear factor kappa B ligand, IL-25 in Asia, and IL-33 in several studies), NFAT (PGD2, cysteinyl leukotriene [LT] C4, and LTD4), STAT5 (TSLP), and STAT6 (IL-13) are all elevated in NPs from T2 CRSwNP.^{105,112-114} This suggests that NP tissue has a potent microenvironment that activates ILC2s to robustly produce T2 cytokines.

Antigen-dependent T2 inflammation is mainly controlled by T_H2 cells. T_H2 cells are differentiated from naive CD4⁺ T cells through activation of T-cell receptor-mediated signaling, costimulation, and STAT6 activation by cytokine signals (IL-4 and IL-13), and GATA3 is a key transcription factor that controls differentiation of T_H2 cells.^{115,116} TSLP also participates in DC-mediated T_H2 differentiation via the induction of OX40L on DCs, and OX40L⁺ DCs are elevated in T2 NPs.^{117,118} Several groups identified the elevation of T_H2 cells in T2 NPs.^{4,80,118-120} T_H2 cells in NPs also express receptor activator of nuclear factor kappa B ligand, which activates ILC2s to produce T2 cytokines.¹¹³ Lam et al¹²⁰ and Ma et al¹²¹ characterized T_H2 cells in T2 NPs by bulk and single-cell RNA sequencing, respectively, and found that these T_H2 cells expressed GATA3, IL-17RB, IL-1RL1 (also known as ST2), HPGDS, and T2 cytokines. This suggests that elevated T_H2 cells in T2 NPs display a pathogenic T_H2-cell phenotype.¹²²⁻¹²⁴ In addition, single-cell RNA-sequencing analysis also found heterogeneity of T_H2 cells in NPs and showed that the other T_H2 subpopulation expressed CD109 but lacked CRTH2. Interestingly, CD109⁺CRTH2⁻ T_H2 cells not only released classical T2 cytokines IL-4, IL-5, and IL-13 but also produced immunosuppressive IL-10 by having a transcription factor FOXP3 in addition

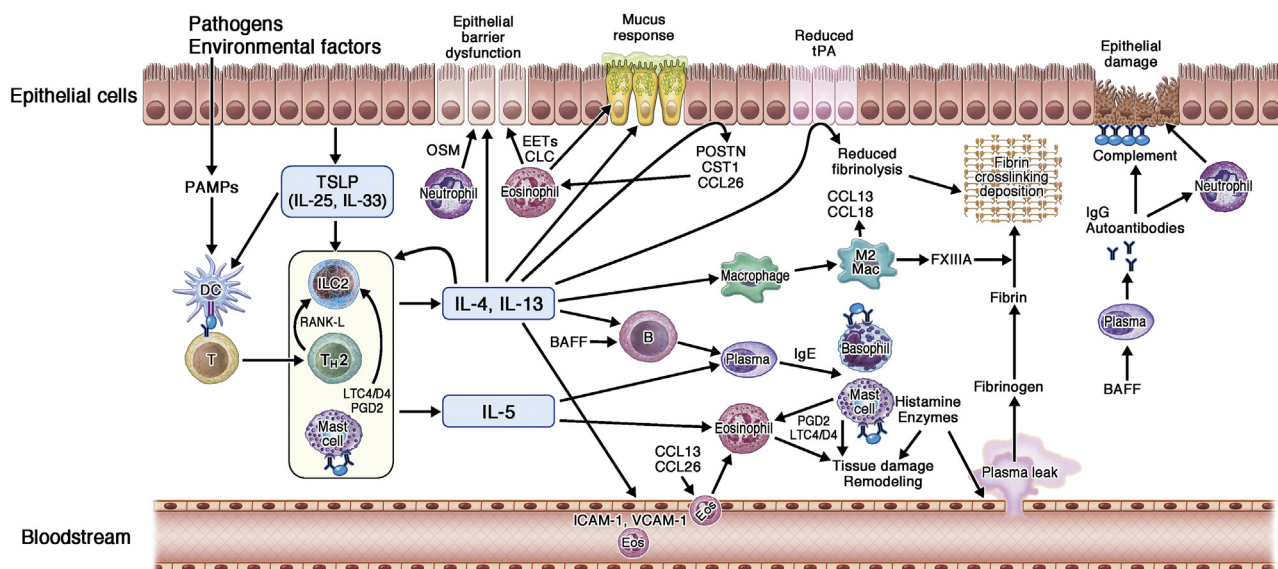


FIG 2. Potential mechanisms in T2 CRSwNP. Pathogens and environmental factors activate nasal epithelial cells, inducing an innate cytokine TSLP. Some countries also found elevation of IL-25 and IL-33. TSLP and PAMPs stimulate DCs to induce naive CD4⁺ T-cell differentiation into Th2 cells. These epithelial-derived innate cytokines also activate ILC2s, pathogenic Th2 cells, and mast cells to produce T2 cytokine. Th2 cells also activate ILC2s to induce T2 cytokines via a RANK-L-dependent pathway. IL-4 and IL-13 activate epithelial cells, endothelial cells, macrophages, and B cells to induce barrier dysfunction, mucus response, eosinophil recruitment, and IgE-mediated reaction. Epithelial dysfunction is also controlled by activation of eosinophils, neutrophils, and complement. Antigen/IgE/IgER complexes on mast cells and basophils induce degranulation and release prestored molecules including histamine and enzymes that induce vascular leak and tissue damage. Plasma leak triggers fibrin deposition in the NPs via cross-linking by FXIIIa released from M2 macrophages and reduction of tPA in epithelial cells. Accumulation of IgG and autoantibodies induces the activation of complements and neutrophils. BAFF, B-cell-activation factor of the TNF family; ICAM-1, intercellular adhesion molecule 1; PAMP, pathogen-associated molecular pattern; RANK-L, receptor activator of nuclear factor kappa B ligand; VCAM-1, vascular cell adhesion molecule 1.

to GATA3.¹²¹ They suggested that CD109⁺CRTH2⁺ Th2 cells might be Tr1 cells that have been primed by T2 microenvironments. Future study will be required to understand the role of CD109⁺CRTH2⁺ Th2 cells in NPs.

B cells and immunoglobulins. Accumulation of B-lineage cells (B cells, plasmablasts, and plasma cells) and elevated local production of immunoglobulins (IgA, IgG, and IgE) are now believed to play a pathogenic role in CRSwNP.^{4,90,125,126} Accumulation of B-lineage cells in NPs was associated with local production of B-cell-activation factor of the TNF family.¹²⁷ Zhang et al⁹⁰ compared immunoglobulin isotypes between T2 and non-T2 NPs in China and found that only IgE was significantly elevated in T2 NPs compared with non-T2 NPs. Elevated IgE plays a pathogenic role in T2 CRSwNP via the activation of mast cells and basophils, and treatment with an anti-IgE antibody (omalizumab) reduced polyp size and improved symptoms in patients with CRSwNP.¹²⁸ IgE antibodies against enterotoxins from *S aureus* were well discussed regarding the pathogenic roles and biomarkers in severe CRSwNP.¹²⁹ High tissue IgE levels also associated with NP recurrence.¹³⁰ Although IgG₄ was similarly elevated in T2 and non-T2 NPs,⁹⁰ IgG₄ was more elevated in severe T2 CRS.¹³¹ Buchheit et al¹³⁰ found that IgG₄ and IgE were further elevated in NPs from patients with aspirin-exacerbated respiratory disease (AERD), which is a respiratory syndrome consisting of severe T2 CRSwNP, asthma, and an intolerance to inhibitors of cyclooxygenase 1 compared with NPs from patients with aspirin-tolerant CRSwNP in the United States. Although whether IgG₄ acts on pathogenic or protective roles in NPs is still

unclear, IgG₄ may be used as a biomarker to predict severe T2 CRSwNP. Interestingly, Buchheit et al¹³⁰ also found IL-5Ra expression on plasma cells in AERD NPs and showed that IL-5 activated plasma cells. This suggests that biologics targeting IL-5 and IL-5Ra not only inhibit eosinophils but also suppress antibody-secreting plasma cells to improve patient outcomes in AERD. Local accumulation of several autoreactive IgG and IgA in NPs such as those targeting double-stranded DNA and basement membrane components has also been reported.¹³²⁻¹³⁴ Levels of autoreactive antibodies in NPs were associated with eosinophil levels and disease recurrence, indicating that elevation of autoreactive antibodies is associated with the T2 endotype. Finally, local accumulation of IgG induces activation of the classical complement pathway in NPs.¹³³ A recent study by Wang et al¹³⁵ showed that PD1^{hi}CXCR5⁺CD4⁺ cells are atypical T cells found in NPs in China, and that these cells purified from T2 NPs potentially activate B-cell production of all immunoglobulin isotypes *in vitro*.¹³⁵

Macrophages. Macrophages are shifted to the M2 phenotype and are elevated in T2 NPs.^{136,137} Elevated M2 macrophages in NPs demonstrated impaired phagocytic activity against *S aureus*, and this may link staph colonization, which is frequently found in T2 CRSwNP.¹³⁶ M2 macrophages are a very important producer of factor XIII-A, which can induce fibrin deposition by cross-linking fibrin that is involved in NP formation (see below).¹³⁸ M2 macrophages also produce CCL13 and CCL18, chemokines that recruit eosinophils and immature myeloid DCs, respectively.^{99,137}

Mast cells and basophils. Mast cells and basophils play key roles in host defense and T2 allergic inflammation.¹³⁹ The primary stimulus for mast cells and basophils in T2 inflammation is activation through the high-affinity IgE receptor FcεRI. Upon cross-linking of FcεRI via IgE-antigen, they release several pre-stored mediators, including histamine and proteases, and synthesize lipid mediators including CysLTs and PGD₂.¹³⁹ They also produce T2 cytokines; in general, mast cells produce IL-5 and IL-13, whereas basophils produce mainly IL-4. Mast cells also respond to cytokines including epithelial-derived IL-33 and TSLP and macrophage/neutrophil-derived IL-1β to produce IL-5 and IL-13.^{3,140} Mast cells are frequently divided into 2 major phenotypes: mast cell-tryptase (MC_T) and mast cell-tryptase/chymase (MC_{TC}). Takabayashi et al¹⁴¹ found that MC_T were elevated in mucosal epithelium and MC_{TC} were elevated in glandular epithelium in the United States. Cao et al¹⁴² found that activated mast cells were elevated in NPs from T2 CRSwNP in China. Dwyer et al¹⁴³ performed single-cell RNA sequencing in NP mast cells and identified 2 more mast-cell subsets: intermediate MC and proliferative MC (Ki67⁺). Within these 4 mast-cell populations, MC_{TC} and intermediate MC showed enhanced expression of chemokines, proinflammatory cytokines, the T2 cytokine IL-13, and a key lipid mediator biosynthetic enzyme PTGS2, suggesting that these mast cell populations are activated and play proinflammatory roles in T2 NPs.¹⁴³ It is also important to note that MC_{TC} in NPs are phenotypically different from MC_{TC} in skin and lung. For example, although dermal MC_{TC} express C5AR1 and Mas-related G protein-coupled receptor X2 and respond to C5a and neuropeptides, respectively, NP MC_{TC} do not have these receptors.¹⁴³ Although elevated MC_T in NPs seem to be activated by IL-4 and/or IL-13, their direct role in NP pathogenesis is still unclear.

In the case of basophils, Stevens et al¹⁴⁴ found that basophils were significantly elevated in NPs from CRSwNP and were further elevated in AERD NPs. Importantly, basophils in AERD NPs were more activated and degranulated and levels of these markers in basophils correlated with sinonasal and pulmonary disease severity.¹⁴⁴ This suggests that basophils also play a role in the pathogenesis of T2 CRSwNP, especially in severe cases.

Eosinophils and neutrophils. Eosinophilia is the central feature in T2 CRSwNP around the world, and biologics targeting eosinophils by anti-IL-5 (mepolizumab and reslizumab) and anti-IL-5 receptor (benralizumab) have been approved or are in clinical trials for treatment of patients with severe T2 CRSwNP.¹⁴⁵ Accumulation of eosinophils in T2 CRSwNP, especially in NPs, is controlled by local elevation of adhesion molecules (vascular cell adhesion molecule 1 [VCAM-1] and intercellular adhesion molecule 1 [ICAM-1]) induced by IL-4 and IL-13, recruitment factors (PGD₂, cysteinyl LTs, and chemokines including CCL13 [MCP4] and CCL26 [eotaxin-3]), as well as survival and activation factors (IL-5 and GM-CSF).¹⁴⁵ Eosinophils in NPs presented increased expression of CD69, which is a marker of activation.¹⁴⁶ Upon activation, eosinophils release pre-stored cytotoxic granule proteins including major basic protein, eosinophil cationic protein, eosinophil-derived neurotoxin, and eosinophil peroxidase, which contribute to tissue damage and remodeling.^{145,147} Activated eosinophils can also exhibit extracellular trap cell death and release eosinophil extracellular traps. Eosinophil extracellular traps are formed with DNA, granule proteins, and Charcot-Leyden crystals (CLCs). CLCs induce proinflammatory cytokines including IL-6, TNF, and

GM-CSF as well as recruitment of neutrophils.^{148,149} CLCs also act as an adjuvant to enhance adaptive T2 immunity.¹⁴⁸ In addition, activated eosinophils synthesize and release cysteinyl LTs. Importantly, NP eosinophils have elevated levels of LTC₄ synthase and γ-glutamyl transferase-5 (converts to LTD₄ from LTC₄) and produce more LTD₄ on activation compared with peripheral blood eosinophils.¹⁴⁶ Released cysteinyl LTs promote further eosinophil recruitment, mucus secretion, vascular permeability, and activation of ILC2s. Finally, eosinophils are a key producer of a chemokine CCL23 that recruits CCR1⁺ DCs, monocytes, and macrophages after undergoing posttranslational modifications in NP tissue.¹⁵⁰

Although neutrophils play a central role in non-T2 CRSwNP, recent studies suggest that neutrophils also contribute to pathogenesis in T2 CRSwNP. Pothoven et al^{20,21} found that the epithelial barrier-disrupting cytokine OSM was elevated in T2 NPs and that OSM was detected in neutrophils in NP tissue. Delemarre et al¹⁵¹ reported the elevation of neutrophils in T2 NPs and found that deposition of CLCs is associated with neutrophil infiltration in Belgium. Subsequently, Poposki et al¹⁵² showed the elevation of activated neutrophils in T2 NPs in the United States, and found that these neutrophils contributed to IL-1β production and were associated with NP recurrence. Succar et al¹⁵³ found that patients with mixed eosinophilic and neutrophilic CRSwNP showed the highest disease symptom scores in the United States. Because the disease severity and recurrence rates are lower in the purely neutrophilic non-T2 NPs than in T2 NPs,⁹⁵ neutrophils may have different pathogenic roles between T2 and non-T2 CRSwNP.

Non-T2 CRSwNP

Although there is heterogeneity in the endotypes of non-T2 CRSwNP such as T1, T3, and neutrophilic, most mechanistic studies did not separate each subendotype. Therefore, we are only able to summarize general non-T2 mechanisms in this review (Fig 3). T cells, B cells, macrophages, and DCs were similarly elevated in both non-T2 and T2 NPs compared with control sinus mucosa; however, the phenotypes of these cells were different between 2 endotypes. Unlike T2 NPs, non-T2 NPs presented elevated levels of IFN-γ, IL-1β, IL-6, IL-8, and IL-17 and accumulation of T_H1 and T_H17 cells.^{5,118} Although activated DCs were elevated in both endotypes, OX40L and PD-L1 were elevated on DCs only from T2 NPs. Macrophages are also shifted to M1 phenotype and produce IL-1β and ROS via the activation of NLRP3 inflammasome.¹⁵⁴ Transcriptome analyses also identified elevated expression of IFN-γ-induced chemokines (*CXCL9*, *CXCL10*, and *CXCL11*), IL-17-induced molecules (*SAA*, *CHI3L1*, *CXCL5*, and *CCL20* [recruits DCs and T_H17]), and neutrophil activators and chemokines (*CSF3*, *CXCL1*, *CXCL2*, *CXCL5*, and *CXCL6*),^{91,92} suggesting that the mixed T1 and T3 endotype with neutrophilia is the common endotype in non-T2 CRSwNP. Neutrophils play an important role in the inflammation associated with non-T2 CRSwNP.¹⁵⁵ Activated neutrophils release pre-stored molecules including myeloperoxidase, elastase, cathepsin G, proteinase 3, and antimicrobial peptides that protect from infected bacteria and induce tissue damage and remodeling. Activated neutrophils also synthesize and produce lipid mediators (mainly LTB₄), ROS, and several cytokines and chemokines including OSM, IL-1β, and IL-8 and contribute to inflammation and further recruitment of neutrophils. Neutrophils also induce NETosis and release neutrophil extracellular traps, and the presence of

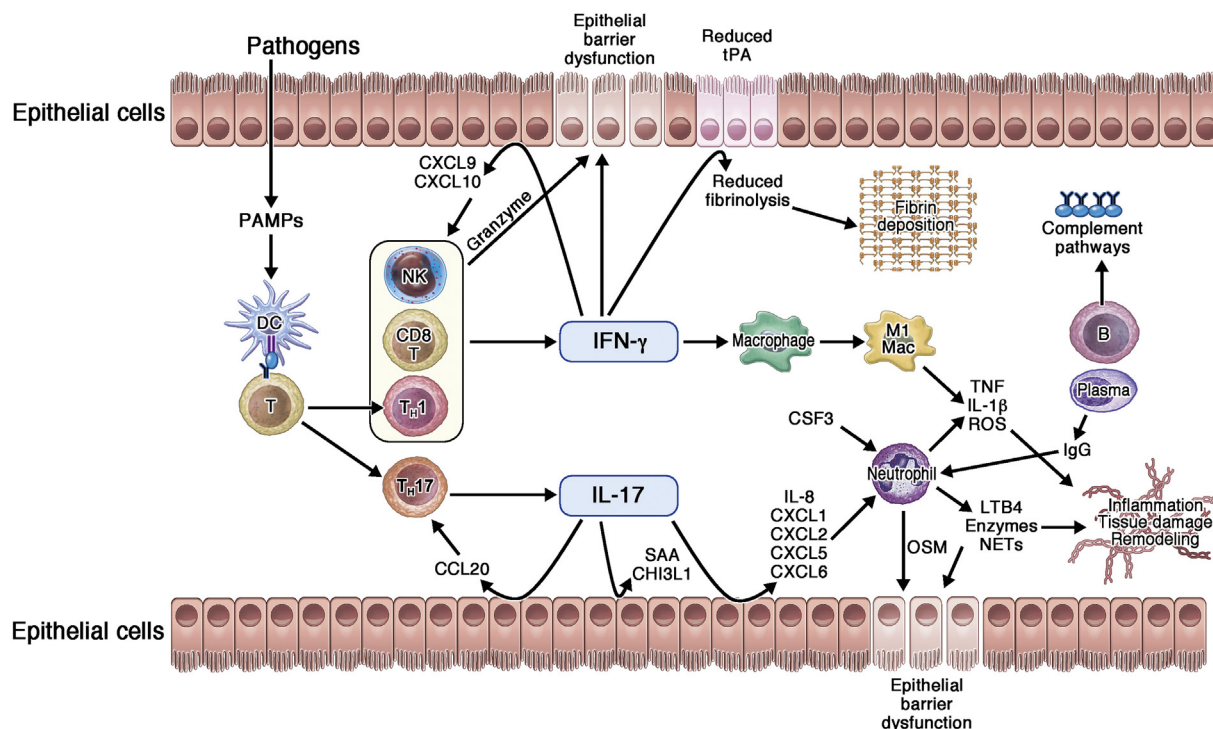


FIG 3. Potential mechanisms in non-T2 CRSwNP. T1-mediated inflammation is mainly triggered by the accumulation and activation of T_H1 cells, CD8⁺ T cells, and NK cells, and IFN- γ is a key cytokine that controls further recruitment of T1 immune cells, epithelial barrier dysfunction, fibrin deposition, and development of M1 macrophages. M1 macrophages produce IL-1 β and ROS to induce inflammation. T3-mediated inflammation is triggered by the accumulation and activation of T_H17 cells. IL-17 induces recruitment of T_H17 cells and neutrophils. Activated neutrophils release OSM, NETs, IL-1 β , LTB4 enzymes, and ROS to induce epithelial barrier dysfunction, inflammation, and tissue damage. IgG antibodies are also elevated in non-T2 CRSwNP and may play a role in the activation of neutrophils and complement pathways. *NET*, Neutrophil extracellular trap; *NK*, natural killer.

neutrophil extracellular traps and positive correlations with neutrophil counts were reported in Chinese NPs.¹⁵⁶

Deposition of fibrin as the main mechanism of hypertrophy in polyps and sinuses

Work by Takabayashi and Schleimer⁶⁵ and Takabayashi et al⁷⁴ revealed widespread deposition of fibrin in NP tissue. Earlier work by Shimizu et al¹⁵⁷ had provided evidence for the presence of active thrombin in nasal lavage fluids from patients with CRS, suggesting activation of the coagulation system. Coagulation is activated both by the intrinsic pathway via surface contact and by the extrinsic pathway via tissue factor; both pathways are likely operative in CRSwNP tissue, and lead to activation of factor X, followed by formation of thrombin and subsequently fibrin.^{158,159} Although it can be expected that any condition or disease that causes vascular leak will promote attendant activation of coagulation and formation of extravascular fibrin clot, ordinarily tPA, which is primarily produced by epithelial cells in the airways, activates plasmin, and degrades the tissue fibrin clot. We now know that the extensive and tenacious deposition of fibrin in CRSwNP is partly attributable to suppression of tPA production and release, resulting in reduced expression of this major fibrinolytic pathway and impeding or preventing clot degradation.^{65,74} Compounding the impact of impaired fibrinolytic pathways, later studies by Takabayashi et al¹³⁸ showed strong induction of factor XIII-A, an important enzyme that crosslinks

and stabilizes fibrin. This factor is expressed by so-called alternatively activated macrophages (M2 macrophages) that are found in high numbers in the tissue of patients with NPs.¹³⁸

Because active coagulation is occurring in the tissue of patients with CRS, it is not surprising that further studies by Imoto et al¹⁶⁰ have found increased levels of 2 pathway members, thrombin-activatable fibrinolysis inhibitor and thrombin/antithrombin complex, in the tissue and nasal fluids of patients with CRSwNP. Interestingly, levels of both thrombin/antithrombin complex and thrombin-activatable fibrinolysis inhibitor were higher in patients with CRSwNP with comorbid asthma, compared with patients with CRSwNP without asthma, suggesting that activation of coagulation is correlated to severity of disease as indicated by asthma comorbidity.¹⁶⁰ Studies by Takabayashi et al in Fukui demonstrated that culture of NP tissue overnight with nattokinase, a fibrinolytic enzyme, led to large-scale shrinkage of polyp tissue but not normal tissue and led them to conclude that most of the wet weight of the polyp tissue (approaching 90%) was attributable to trapping of water by deposition of fibrin mesh. This important study strongly suggests that polyp formation itself is to a large extent driven by deposition of large quantities of fibrin. Interestingly, in the same study, they showed that experimental degradation of fibrin in mucus led to nearly a complete loss of mucus viscosity, suggesting that crosslinked fibrin is also found in nasal mucus and endows mucus with many of its elastic and adhesive properties.¹⁶¹ The initial studies referred to above demonstrated fibrin deposition using

immunohistochemistry or immunofluorescence.⁷⁴ More recently, unpublished studies at Northwestern using a biochemical assay to quantitate fibrin deposition have demonstrated a 10- to 20-fold increase in fibrin protein in NP tissue compared with normal ethmoid or uncinat tissue. Interestingly, hypertrophic tissue from the ethmoid sinuses of patients with CRSsNP was also found to contain fibrin mesh elevated to nearly the same level as seen in NP tissue.

Mechanisms by which the coagulation pathway mediates fibrin deposition and subsequent formation and maintenance of polyp and hyperplastic tissues have been discussed elsewhere.⁶⁵ Several studies suggested that T2 inflammation drives the fibrin deposition in polyps. For example, the extent of fibrin deposition correlates with T2 biomarkers; the formation of alternatively activated macrophages in the tissue, and their production of the fibrin cross-linking molecule factor XIII-A, also correlated with T2 biomarkers; *in vitro* studies with epithelial cells demonstrated that exposure to IL-4 or IL-13 suppressed the expression of tPA; levels of secondary markers of coagulation also correlated with T2 biomarkers; expression of L-plastin in polyp eosinophils correlated with T2 responses.^{65,74,138,160} These findings, although implicating T2 cytokines in activation of coagulation and fibrin deposition, do not explain why there are many patients in China with noneosinophilic disease along with formation of NPs. To study this, Chen et al evaluated these questions in China and found that fibrin was increased and tPA was decreased in the tissue of both eosinophilic polyps and noneosinophilic polyps. Thrombin/antithrombin complex was also increased in both endotypes of patients. Finally, *in vitro* studies showed that T1 and T3 cytokines could also decrease tPA.¹⁶² These studies suggest that activation and prolongation of coagulation and fibrin deposition are not events related strictly to T2 inflammation.

It is worthwhile pointing out that suppression of coagulation by topical or systemic drugs has the potential to promote fibrinolysis and shrink NPs regardless of endotype. Imoto et al¹⁶³ have shown that short-chain fatty acids (SCFAs) such as propionic acid or butyric acid, as well as retinoic acid, are active in inducing the expression of tPA in epithelial cells, so it may be worthwhile to attempt local delivery of a topically bioavailable retinoic or other SCFA in a trial. SCFAs induce tPA in airway epithelial cells via GPR41&43.¹⁶³ Trials of anticoagulants, either locally or systemically, may also be worthwhile to determine whether these drugs are capable of shrinking NP tissue. Studies suggest that nattokinase, a fibrinolytic enzyme used as a food supplement, and SCFAs both have potential to be used in such an application.^{161,163}

INFLAMMATION IN CRSsNP

Although CRSsNP was initially classified as T1 inflammation based on the elevation of IFN- γ ,^{4,6} recent studies could not confirm overall elevation of IFN- γ in CRSsNP.⁷⁻⁹ There are several possibilities for this. One possibility is that there has been an inflammation shift toward T2 away from T1 as has occurred in Asian NPs. In addition, studies in CRSsNP have been complicated by the use of variable sinonasal biopsy sites having inherent tissue-specific molecular differences.¹⁶⁴ By the exclusive use of ethmoid sinus mucosa, it is now clear that inflammatory endotypes in CRSsNP are highly heterogeneous and the frequency of each endotype varies geographically.⁷⁻⁹ The T2 endotype has become most common in Western countries, and 30% to 55% of patients with CRSsNP present the T2 endotype. In

contrast, T1 is still a predominant endotype in Asian CRSsNP, although 30% to 40% of patients with CRSsNP in Beijing (China) also have T2 and/or T3 endotypes.⁷ However, most patients with CRSsNP in Chengdu (China) did not show the elevation of any endotypic (T1, T2, or T3) markers. Currently, heterogeneity and geographical differences introduce difficulty studying the pathogenic mechanisms in CRSsNP.

On the basis of these limitations, Klingler et al¹⁶⁵ used preendotyped ethmoid sinus mucosa in CRSsNP and performed microarray analysis. This study identified T1, T2, and T3 endotype-specific genes expressed in CRSsNP that have been useful in developing an understanding of the underlying molecular mechanisms of each endotype in CRSsNP. Based on the gene expression profile, T1 CRSsNP is characterized by the accumulation of T_H1 cells, CD8⁺ cytotoxic T cells, natural killer cells, and antigen-presenting cells. The release of IFN- γ and cytotoxic molecules including granzymes (*GZMA*, *GZMB*, and *GZMH*) may play key pathogenic roles in T1 CRSsNP. T2 CRSsNP is characterized by the elevation of T2 cytokines *IL-4*, *IL-5*, and *IL-13*, markers of T2 immune cells including T_H2 cells, ILC2s, eosinophils, mast cells, basophils, DCs, and M2 macrophages, and other common T2 markers including *CCL18*, *CCL26*, *POSTN*, and *CST1*.¹⁶⁵ Markers of regulatory T cells (*FOXP3* and *IL10*) were also elevated in T2 CRSsNP, and this may be due to the presence of conventional regulatory T cells as well as CD109⁺CRTH2⁻ T_H2 cells that are found in T2 NPs.¹²¹ In addition, this study clarified that T2 CRSsNP-specific genes were also elevated in T2 CRSwNP, suggesting that the mechanisms of T2 inflammation in T2 CRSsNP and T2 CRSwNP are very similar. T3 CRSsNP seemed to be characterized by the accumulation of T_H17 cells, B cells, DCs, M1 macrophages, and neutrophils. Activation of neutrophils and complement pathways, and production of proinflammatory cytokines TNF and IL-1 β from macrophages and neutrophils, may play key pathogenic roles in T3 CRSsNP. Finally, it is important to note that a significant proportion of patients with CRSsNP did not display a visible elevation of any T1, T2, or T3 endotypic markers in the ethmoid sinus mucosa across the world.^{7-9,166} There are many potential explanations for this: these patients may have mild elevation of endotypic markers but do not permit classification based on the thresholds; they may have T1, T2, or T3 inflammation in other sinonasal mucosae; or they may have unrecognized endotypes besides T1, T2, and T3.¹⁶⁶ To understand pathogenic roles of CRSsNP, future studies will require larger study populations with consideration of these possibilities and their pathogenic mechanisms.

CONCLUSIONS

The realization that CRSsNP and CRSwNP are both heterogeneous diseases at a molecular level has been exciting, informative, and challenging. There are still several key areas that we will need to study on CRS (Table I). For physicians, it means that the variable efficacy of the various treatments used may result in part from heterogeneous immunopathology. It means outcomes may vary. The use of biologics, though approved, may be unreliable because these T2-targeting drugs will be inadvertently administered to patients with non-T2 disease. Although good practice of medicine and collection of information on the presence of eosinophils and pus can give physicians a good basis to extrapolate endotype, we are not yet near the stage where physicians treating CRS will have ready access to laboratory assays for endotyping to

TABLE I. Items that we will need to further investigate in CRS

What is known	Questions and gaps in understanding
Three inflammatory endotypes (T1, T2, and T3) are present in CRS ^{7-9,166}	Why do only patients with CRSwNP develop NPs even when both patients with CRSsNP and patients with CRSwNP have similar inflammatory conditions? Are there additional endotypes besides T1, T2, and T3 in CRS, especially in CRSsNP? We need to identify biomarkers and systems that can determine endotypes in a clinical situation
Biologics targeting T2 endotype including IL-4Ra, IL-5, IL-5R, IgE, and TSLP have been approved or are in clinical trials in CRSwNP ^{2,97,108,128,145}	Determine criteria by which a physician selects a particular biologic for each patient with CRSwNP Determine whether T2 biologics are effective in patients with CRSsNP who have the T2 endotype Develop new treatment strategies for non-T2 CRS
Biologic and systemic corticosteroid responders demonstrate relapsing inflammation following withdrawal of therapy ^{2,97,167,168}	What are the mechanisms that drive relapsing T2 inflammation even in the setting of complete pharmacologic responders?
A subset of patients with CRS has a mixed inflammatory endotype ^{7-9,166}	Do patients with CRS with mixed endotypes develop additional unique pathogenic factors?
TSLP is elevated in T2 NPs ^{104,105}	The presence and roles of IL-25 and IL-33 in T2 NPs are still largely unclear
MC _{TC} play a role in pro- and T2-inflammation in NPs ¹⁴³	The role of elevated MC _T in NPs in T2 CRSwNP is still unclear
IgG ₄ is elevated in severe CRSwNP and AERD ^{130,131}	The role of IgG ₄ in NPs is not clear
Persistent overexpression of epithelial-derived protease inhibitors differentiates T2 CRS from transient upregulation in seasonal allergic rhinitis ^{63,64}	How does downregulation of epithelial function in T2 CRS contribute to the initiation and maintenance of inflammation?

inform clinical decision making. But it has expanded the possibility that many patients with CRSsNP, whose disease was previously thought to be non-T2, may benefit from T2-targeting drugs. Patients with T3 and T1 cytokines may be suffering from inapparent infections with bacteria and fungi, or viruses, respectively, and therefore not responsive to any anti-inflammatory treatments. For investigators, the exciting new data open doors to improve patient care. If new accessible, rapid assays for endotyping can be incorporated into the clinic, choice of treatments and expectations of outcomes can be improved. These assays can also inform large, prospective clinical, epidemiological, or genetic studies to teach us the impact of endotype on severity, comorbidities, outcomes, and many other important clinical parameters. We hope that this review stimulates the imaginations of physicians and investigators alike in our collective drive to improve the care of patients with CRS in all of its many forms.

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