Laryngoscope Investigative Otolaryngology

Innate Immunity and Chronic Rhinosinusitis: What We Have Learned From Animal Models

Nyall R. London Jr., MD, PhD; Andrew P. Lane, MD

Objective: Chronic rhinosinusitis (CRS) is a heterogeneous and multifactorial disease characterized by dysregulated inflammation. Abnormalities in innate immune function, including sinonasal epithelial cell barrier function, mucociliary clearance, response to pathogen-associated molecular patterns (PAMPs) via pattern recognition receptors, and the contribution of innate immune cells, will be highlighted in this review.

Data Sources: PubMed literature review.

Methods: A review of the literature was conducted to determine what we have learned from animal models in relation to innate immunity and chronic rhinosinusitis.

Results: Dysregulation of innate immune mechanisms, including sinonasal barrier function; mucociliary clearance; PAMPs; and innate immune cells such as eosinophils, mast cells, and innate lymphoid cells, may contribute to CRS pathogenesis. Sinonasal inflammation has been studied using mouse, rat, rabbit, pig, and sheep explant or in vivo models. Study using these models has allowed for analysis of experimental therapeutics and furthered our understanding of the aforementioned aspects of the innate immune mechanism as it relates to sinonasal inflammation. These include augmenting mucociliary clearance through activation of the cystic fibrosis transmembrane conductance regulator and study of drug toxicity on ciliary beat frequency. Knockout models of Toll-like receptors (TLR) have demonstrated the critical role that these pattern recognition receptors play in allergic inflammation because loss of TLR2 and TLR4 leads to decreased lower airway inflammation. Mast cell deficient mice are less susceptible to ovalbumin-induced sinonasal inflammation.

Conclusion: Animal models have shed light as to the potential contribution of dysregulated innate immunity in chronic sinonasal inflammation.

INTRODUCTION

Chronic rhinosinusitis (CRS) remains a challenging disease to treat. The direct and indirect costs are substantial, with approximately $8.3 billion spent annually, significant associated lost job productivity, and poor health-related quality of life.[1] This disease is defined by greater than 12 weeks of a combination of facial pressure, anosmia, mucopurulent nasal drainage, and nasal obstruction.[1–3] Although these symptoms have a high sensitivity, objective evidence of CRS via nasal endoscopy or computed tomography (CT) imaging is necessary due to the poor specificity of the aforementioned symptoms.[4] Common treatment options include oral or intranasal steroids, saline rinses, and antibiotics, with refractory cases progressing to surgery and continued medical therapy thereafter.

Chronic rhinosinusitis is broadly divided into CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). CRSwNP is characterized by the presence of polyps and an eosinophilic inflammatory infiltrate, whereas CRSsNP is characterized by noneosinophilic inflammation associated with neutrophil accumulation, tissue remodeling, and fibrosis.[3] Patients suffering from CRSwNP typically complain of nasal obstruction by the nasal polyps, anosmia, and congestion, whereas CRSsNP may have similar symptoms but are more likely to also report facial pain or headache.[5] Within these two divisions, significant pathophysiologic diversity exists.[6] Attempts at further subdivision based on histopathology, expression profiles, and clinical criteria have suggested the presence of multiple clusters or endotypes.[3,6] The variety of histopathologic features and severity, as well as the variability of tissue markers such as albumin, IgE, and interleukin-5, underline the fact that CRS is highly heterogeneous and multifactorial.[3,6]

At the center of CRS is dysregulated persistent inflammation, which may be perpetuated by both innate and adaptive immune mechanisms. The innate immune system does not depend on previous exposure and provides the initial inflammatory antimicrobial response.[7] The epithelial cell layer contributes greatly to sinonasal innate immunity through physical barrier function, mucociliary clearance, and secretion of antimicrobial products. Epithelial cells recognize pathogen-associated and damage-associated molecular patterns (PAMPs and DAMPs) via pattern recognition receptors (PRRs) including toll-like receptors (TLRs),

From the Department of OtolaryngologyHead and Neck Surgery, Johns Hopkins School of Medicine, (N.R.L., A.P.L.), Baltimore, Maryland, U.S.A.

Editor's Note: This Manuscript was accepted for publication 10 May 2016.

Conflict of interest: N.R.L. has stock in Navigen Pharmaceuticals that is currently of no value. The authors have no other funding, financial relationships, or conflicts of interest to disclose.

Send correspondence to Andrew P. Lane, MD, Johns Hopkins Outpatient Center, 601 N. Caroline Street, 6th Floor, Baltimore, MD, 21287-0910. E-mail: alane3@jhmi.edu

DOI: 10.1002/lio2.21

nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), retinoic acid-induced gene (RIG)−1–like receptors (RLRs), and extraoral taste receptors. Activation of epithelial cell PRRs and cytokine receptors induces apical secretion of antimicrobials and basal production of signaling molecules that interact with infiltrating inflammatory cells, such as granulocytes, macrophages, and lymphocytes, while also communicating with resident cell types, such as dendritic cells, mast cells, fibroblasts, and innate lymphoid cells (ILCs).[7–9] Both innate and adaptive immune responses are critical to defense against infection and injury and must be coordinated to maintain homeostasis and resolve inflammation. A persistent T-helper 2 (Th2)-skewed inflammatory state has been associated with CRSwNP in Western populations, characterized by local tissue presence of IL-5, IgE, and abundant eosinophils.[7]

The relative accessibility of sinonasal mucosa tissue has permitted descriptive studies of innate immunity in health and chronic sinus inflammatory disease, including expression of genes and mediator proteins, histopathologic features, and flow cytometric analysis of cell populations. Although in vitro techniques of human epithelial cell cultures and explants provide models for experimental investigation of innate immune mechanisms, animal models allow in vivo manipulations that are not possible in human subjects, in particular the studies of targeted genetic modifications and investigational therapeutics. However, because of significant anatomic and physiologic differences between animals and humans, animal models must be selected carefully and findings must be cautiously interpreted. There is no animal that naturally develops CRS. Although mice reproduce quickly and are amenable to genetic modification, they lack human-like paranasal sinuses. Thus, whereas specific scientific questions that are difficult to address in humans can be approached with animal models, a global understanding of the heterogeneous and multifactorial disease of CRS in humans cannot be fully recapitulated or understood. Models of sinusitis include acute infection with bacteria or nasal allergen sensitization.[10] These techniques induce particular forms of sinonasal inflammation that can be studied to provide insights into mucosal immunity. The allergy sensitization model generates Th2 inflammatory cytokines and eosinophil accumulation and may result in polyp-like changes reminiscent of CRSwNP.[2,11,12] In this review of innate immunity and CRS, we will focus on insights provided by animal model research (Table I).

Sinonasal Epithelium: A Physical Barrier

Akin to other epithelial cell layers throughout the body, the sinonasal epithelium provides a physical barrier to the external environment. Indeed, epithelial barrier dysfunction is a proposed pathogenic mechanism in chronic inflammatory conditions including Crohn's disease, asthma, atopic dermatitis, and psoriasis.[13] Intercellular adhesion molecules are essential for maintaining barrier function. These include epithelial cadherin (E-cadherin) and tight junction proteins such as junction adhesion molecule, claudin, occludin, and zonula occludens.[3,14,15] Interestingly, protocadherin-1 (PCDH-1), which is a member of the cadherin superfamily, has been identified as a susceptibility gene in bronchial hyperresponsiveness and eczema in human genetic studies.[16,17] Protocadherin-1 expression is increased during airway epithelial cell differentiation and barrier development and colocalizes with E-cadherin in airway epithelial cells in vitro.[18,19] Knockdown of PCDH-1 expression eradicated stabilization of the airway epithelial barrier by dexamethasone in vitro.[19] Thus, there is precedent linking epithelial barrier dysfunction to airway inflammation.

Reduced expression of junction adhesion molecules has been demonstrated in tissue from CRS patients and thus is suggestive of epithelial barrier breakdown.[19,20] However, there is insufficient evidence demonstrating that epithelial barrier destabilization precedes and is causative of CRS. Disruption of sinonasal epithelial barrier function has been proposed to contribute to CRS through allowing increased and chronic exposure of the underlying tissue to inflammatory stimuli.[15] Inflammatory stimuli that have been reported to disrupt airway epithelial cell barrier function in vitro include interleukin-4 (IL-4), interleukin-13 (IL-13), tumor necrosis factor-α (TNF-α), interferon-γ, and house dust mite antigen.[21] Few studies, however, have reported direct measurement of sinonasal epithelial barrier permeability in animal models of CRS. In mice, a recent study directly investigated barrier function in a house dust mite-induced model of allergic rhinitis.[22] Interestingly, an approximately twofold increase in permeability was noted in mice challenged with intranasal house dust mite antigen, and this effect was significantly improved with treatment of fluticasone. Future studies are necessary to further understand the role of sinonasal barrier dysfunction in CRS.

Mucociliary Clearance

The sinonasal cavity is constantly exposed to environmental stimuli including particulate matter, allergens, and microbes.[23] Mucociliary clearance is a key first-line defense against potential airborne threats. Effective clearance is dependent on effective ciliary beating, as well as control of the airway surface liquid, through mucus secretion and active and passive ion transport.[24] The viscous mucus layer glides over the thinner periciliary liquid layer and thereby mobilizes particles.[23] The viscous layer is composed in part by glycosylated high molecular weight molecules derived from mucin genes *MUC5AC* and *MUC5AB*.[23] Cilia beat in a coordinated fashion known as metachronal wave to direct mucus and debris toward the sinus ostia and ultimately to the nasopharynx or oropharynx.[4,25,26] This process is stimulated by multiple allergens, microbes, and irritants, but may also be modulated by signaling mechanisms including cholinergic, adrenergic, and neuropeptide mediators.[4,23,26–30]

The importance of effective mucociliary clearance in maintaining sinus health is demonstrated in patients with genetic or acquired defects in ciliary function, such as primary ciliary dyskinesia or toxic injury, or abnormal mucus as seen in cystic fibrosis (CF).[30,31] The cystic fibrosis transmembrane conductance regulator (CFTR)

TABLE I.
Examples and Insights Gained From Animal Models of Sinonasal and Airway Allergic Inflammation.

Epithelial Barrier Function		
Guinea pig		
1989	Burns et al.[79]	Guinea pigs exposed to cigarette smoke demonstrated increased epithelial barrier permeability to FITC-dextran.
Mouse		
2015	Yang et al.[80]	*Stard7*$^{+/-}$ mice demonstrated exaggerated allergic responses and epithelial barrier permeability in the lung.
2016	Steelant et al.[22]	House dust-mite antigen administered intranasally increased epithelial barrier permeability to FITC-dextran, which was significantly improved with treatment of fluticasone.
Rat		
2007	Tillie-Leblond et al.[81]	Keratinocyte growth factor administration improved epithelial barrier permeability to radiolabelled iodine in ovalbumin sensitized rats.
Mucociliary Clearance		
Mouse		
2013	Liu et al.[82]	*Splunc1*$^{-/-}$ mice demonstrated accelerated mortality to *P. aeruginosa* infection and increased biofilm formation.
2015	Woodworth[24]	Nasal septal epithelial cultures in vitro and nasal potential difference in vivo was used to investigate resveratrol-induced CFTR activation.
	Illing et al.[32]	Nasal septal epithelial cultures in vitro from *CFTR*$^{+/+}$ and *CFTR*$^{-/-}$ mice were used to investigate chlorogenic acid-induced CFTR transport.
Pig		
2012	Chang et al.[34]	Transgenic CF pigs with CFTR mutations develop phenotypes more closely resembling humans because older CF pigs demonstrate spontaneous sinusitis not present at birth.
2014	Dean et al.[33]	Nasal septal epithelial cultures demonstrated similarities to human respiratory epithelia not demonstrated in murine cells.
Rabbit		
2009	Tamashiro et al.[42]	Exposure to a harsh antimicrobial rinse intended to disrupt biofilms was found to significantly reduce ciliary beat frequency in rabbit septal explants.
Sheep		
2013	Boase et al.[83]	Sinonasal fungal biofilm formation occurred in the presence of cilia toxin.
Pattern Recognition Receptors		
Mouse		
2002	Hussain et al.[50]	Treatment with the TLR9 agonist CpG during ovalbumin sensitization abrogated nasal symptoms, decreased upper airway, eosinophilic inflammation, and decreased IL-4 and IL-5 cytokine levels
2009	Hammad et al.[47]	TLR4 expression in structural lung cells was found to be necessary for house dust mite-driven allergic airway inflammation.
2013	Wu et al.[51]	Prophylactic vaccination with monophosphoryl lipid A, a TLR4 agonist, was shown to reduce airway hyperresponsiveness, eosinophilic inflammation, and Th2-mediated responses.
2014	Li et al.[46]	TLR2 knockout mice exhibited less airway hypersensitivity, inflammation, and Th2 cytokine levels in an OVA model of allergic asthma.
	Lee et al.[58]	Innate immune function of extraoral taste receptors was found to be conserved across humans and mice, and an unexpected murine α-gustducin-independent mechanism was uncovered.
Innate Immune Cells		
Mouse		
2000	Lambrecht et al.[61]	Ovalbumin inhalation significantly increased inflammatory cell accumulation and Th2 cytokines in mice administered intratracheal OVA-pulsed myeloid dendritic cells.
2011	Chang et al.[71]	Depletion of ILC2s with anti-Thy1.2 antibody results in reduced airway hypersensitivity after influenza infection
2015	Murakami et al.[60]	Dendritic cell subset manipulation toward Th1 response decreased OVA-induced nasal inflammation
2016	Hua et al.[68]	C57BL/6-Kit$^{W\text{-}sh/W\text{-}sh}$ mast cell-deficient mice subjected to intranasal OVA once per week for 12 weeks did not develop nasal polyps.
Microbial Infection		

TABLE I.
(*Continued*)

Ferrett		
2006	Peltola et al.[84]	Sinusitis was observed in young ferrets infected with H3N2 influenza A followed by *S. pneumonia*.
Rabbit		
2009	Chennupati et al.[85]	Antimicrobial peptide activity and toxicity were evaluated in a rabbit model of *P. aeruginosa* biofilm formation.
Sheep		
2011	Boase et al.[86]	Sinonasal fungal biofilm formation only occurred *S. aureus* coinoculation
2014	Drilling et al.[87]	Topical bacteriophage and EDTA treatment was efficacious against *S. aureus* infection in a sheep sinusitis model.

This table highlights the mechanistic insights that have been gained when employing genetic deletion and preclinical therapies in animal models of allergic inflammation. Areas of interest in innate immunity have included epithelial barrier function, mucociliary clearance, pattern recognition receptors, and innate immune cells.

CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; IL- = interleukin; ILC = innate lymphoid cell; OVA = ovalbumin; Th2 = T-helper 2; TLR = Toll-like receptors.

mediates transepithelial Cl^- transport, a critical step in regulating the viscosity of the ASL. In humans with mutations in the CFTR, mucous secretions become thick, resulting in poor mucociliary clearance and near universal CRS.[32] As a single-locus genetic disease, CF is an attractive condition to model by genetic manipulation in a laboratory animal. Although CFTR mutations have been generated in mice, the outcome did not mirror the manifestations of CF in humans. In contrast, transgenic CF pigs with CFTR mutations develop phenotypes more closely resembling humans because older CF pigs demonstrate spontaneous sinusitis not present at birth.[33,34] Other patients may have deficiencies in ciliary function or structure. These may be congenital, such as primary ciliary dyskinesia, or acquired defects secondary to toxins or inflammatory stimuli.[24,26,35]

A proposed mechanism whereby poor mucociliary clearance contributes to CRS is through stasis and subsequent chronic exposure to microbes and inflammatory stimuli.[4] Poor mucociliary clearance is a common finding in CRS.[4,36] Interestingly, ciliary beat frequency is not significantly different between CRS and control patients at baseline but does demonstrate a blunted response to substances that stimulate ciliary activity.[4,37,38] Potentially contributing to this finding are toxins secreted by bacteria such as *S. aureus* and *P. aeruginosa*, which directly suppress ciliary activity.[4] Chronic exposure to inflammatory cytokines such as TNF-α may also blunt an appropriate ciliary response. Thus, deficiency in mucociliary clearance may contribute to CRS through a cycle of microbial colonization and chronic inflammation. Identification of ways to improve mucociliary clearance may have clinical benefit in CRS patients.[36]

Although acquired defects in the CFTR may contribute to poor mucociliary clearance in human disease, augmenting the function of the CFTR to increase mucociliary clearance has been proposed as a potential therapeutic target and investigated using animal models.[32,39] Indeed, the small molecule resveratrol-stimulated CFTR-dependent Cl^- ion secretion across the nasal epithelium in vivo, as assessed by nasal transepithelial potential difference.[24,40] Nasal septal epithelial cell cultures derived from mice can be a powerful model given the technology for targeted genetic manipulation. Using this system, the Cl^- secretion initiated by resveratrol was demonstrated to be through the CFTR because this effect was lost in nasal septal epithelial cells harvested from $CFTR^{-/-}$ mice.[24] Ciliary beat frequency can be studied in a septal explant, which can be useful to evaluate ciliary toxicity of potential therapeutic approaches.[27,41] Indeed, exposing rabbits to a harsh antimicrobial rinse intended to disrupt biofilms was found to significantly reduce ciliary beat frequency in rabbit septal explants.[42,43] Thus, animal models are useful both to study mechanism of action through genetic modifications but also to evaluate for potential unintended ciliary toxicity, which could worsen CRS.

Pathogen-Specific Mechanisms: A Response to Bacterial Byproducts

Another mechanism proposed to contribute to CRS is the innate immune response to PAMPs, which correspond with bacterial, fungal, and viral pathogens.[44] These are recognized by germline-encoded pattern recognition receptors (PRRs), including (TLRs), NLRs, extraoral taste receptors, and RLRs.[3] Although these receptors allow a degree of pathogen specificity, they are not tailored to antigens by genetic recombination such as receptors and antibodies of the adaptive immune system.

Toll-like receptors are perhaps the best characterized PRR. They are typically expressed on inflammatory cells and cells exposed to the external environment and include macrophages, dendritic cells, endothelial cells, and epithelial cells.[44,45] These receptors consist of an extracellular domain with leucine-rich repeats and an intracellular signaling domain similar to the interleukin-1 receptor family (TIR domain). Important downstream-signaling events leading to inflammatory cytokine secretion include the myeloid differentiation primary response 88 and nuclear factor-κB.[3,44] alterations in expression of TLR2, TLR4, and TLR9 have been described in CRS,[21] and cytokines have been demonstrated to modulate TLR expression and function in human epithelial cells. The use of genetic tools in animal

models has allowed investigation of the role of TLR in chronic airway inflammation. Toll-like receptors knockout mice have been studied in allergic asthma, and the understanding of TLR deficiency in allergic lower airway inflammation is greater than in sinusitis models. Toll-like receptors-2 knockout mice exhibit less airway hypersensitivity, inflammation, and Th2 cytokine levels in an ovalbumin (OVA) model of allergic asthma.[46] The role of TLR4 was investigated in irradiated TLR4 knockout mice in which the bone marrow was reconstituted with cells from either wild-type or TLR4 knockout mice. Th2 cytokine production was abolished in TLR4-deficient mice and did not recover with wild-type bone marrow chimera, demonstrating that TLR4 expression in structural lung cells is necessary for house dust mite-driven allergic airway inflammation.[47]

Activation of TLRs has been suggested in mouse models as a potential therapeutic strategy to shift Th2-skewed inflammation back toward Th1.[48] New agents based on animal research have led to clinical trials to treat allergic rhinitis and asthma.[49] In a mouse nasal allergy model, treatment with the TLR9 agonist CpG during ovalbumin sensitization abrogated nasal symptoms, decreased upper airway eosinophilic inflammation, and decreased IL-4 and IL-5 cytokine levels.[50] In an allergic asthma model, prophylactic vaccination with monophosphoryl lipid A—a TLR4 agonist—has been shown to reduce airway hyperresponsiveness, eosinophilic inflammation, and Th2-mediated responses.[51]

A second group of PRRs proposed to participate in innate immunity and modulate CRS include extraoral taste receptors. T2R bitter and T1R sweet taste receptors are found in multiple sites outside of the oral cavity, including the respiratory system.[52,53] These receptors function as PRRs and have been demonstrated to be expressed on both ciliated respiratory cells as well as solitary chemosensory cells.[53–55] In respiratory cells, T2Rs are located in motile cilia, and ciliary beating is increased upon activation by bitter agonists.[52] In addition to increased ciliary beating, activation of T2R38 in sinonasal epithelial cells stimulates rapid release of nitric oxide, which may itself or through downstream events possess antibacterial activity.[56] In human genetic studies, the single-nucleotide polymorphism TAS2R38 I296V has been reported and replicated to be associated with chronic rhinosinusitis.[57]

Although mice lack the T2R38 receptor, they have been reported to respond to T2R38 agonists. Harnessing knockout mice for proteins in canonical taste-signaling pathways, including TRPM and α-gusducin, one group sought to further investigate the response of mice to taste agonists.[58] In the utilization of these genetic systems to study the downstream signaling effects of acyl homoserine lactone quorum-sensing molecules, an unexpected α-gustducin-independent mechanism was uncovered.[58] These studies further demonstrated that the innate immune function of extraoral taste receptors is conserved across humans and mice. Collectively, there are a multitude of PRRs that likely serve overlapping and redundant roles in innate immune responses but may contribute to the multifactorial disease process of CRS.

Innate Immune Cells: Contributors to Dysregulated Sinonasal Inflammation

Innate immune effector cells, including dendritic cells, mast cells, eosinophils, and ILCs, may contribute to CRS pathophysiology.[59] Dendritic cells phagocytose and present antigens to lymphocytes and can aid in skewing the secretory phenotype of Th1, Th2, Th17, and regulatory T cells, as well as B cells. The details of adaptive immunity are beyond the scope of this review, but multiple innate immune cell types contribute to the transition from rapid innate immune responses to sustained and chronic inflammation. In Th2-skewed inflammation, Th2 lymphocytes are a major source of IL-4, IL-5, and IL-13. In mice, two subsets of dendritic cells have been identified: CD205+ dendritic cells (which elicit a Th1 polarization) and 33D1+ dendritic cells (which shift toward a Th2 phenotype).[60] Utilizing an OVA-induced allergic nasal inflammatory model, this group hypothesized that shifting the dendritic response toward a Th1 subset would modulate allergic symptoms. Through the depletion of 33D1+ dendritic cells using an anti-33D1 antibody, as well as selective activation of CD205+ dendritic cells using the glycolipid antigen α-GalCer, they reported a decrease in nasal symptoms such as rubbing and sneezing, as well as a decrease in inflammatory cell accumulation in nasal lavage fluid.[60] The role of dendritic cells has been much more extensively studied in animal models of airway inflammation compared to sinonasal inflammation. These techniques include adoptive transfer as well as dendritic cell depletion. One landmark study transferred ovalbumin-pulsed myeloid dendritic cells intratracheally to mice and demonstrated, upon subsequent ovalbumin inhalation, significantly increased inflammatory cell accumulation and Th2 cytokines compared to control pulsed myeloid dendritic cells.[61] Interestingly, myeloid dendritic cells have been reported to be increased in CRSwNP, as assessed by flow cytometry and immunohistochemistry.[62–64] In contrast to plasmocytoid dendritic cells, myeloid dendritic cells are believed to be responsible for antigen presentation to T cells and subsequent T-cell polarization.[59] Dendritic cells are known to secrete multiple cytokines and chemokines including eotaxin; thus, dendritic cells in nasal polyps may also contribute to the accumulation of additional inflammatory cells types.[59]

Mast cells have been demonstrated to play a role in host defense and allergic inflammation.[59] Upon recognition of IgE, Fc receptors are activated and mast cells release histamine and proteases. Increased mast cells have been reported in allergic and nonallergic eosinophilic fungal sinusitis patients, as well as a nonstatistically significant increase in CRS patients.[65] Furthermore, high tryptase levels have been reported in nasal secretions from subjects with CRSwNP.[66,67] Although these studies demonstrate an association, Hua et al. turned to a mouse model to further study the contribution of mast cells in a mouse model of CRS.[68] To do so, they utilized C57BL/6-Kit$^{W\text{-}sh/W\text{-}sh}$ mast cell-deficient mice and subjected these mice to intranasal OVA once per week for 12 weeks. Although 67% of wild-type mice exposed to OVA

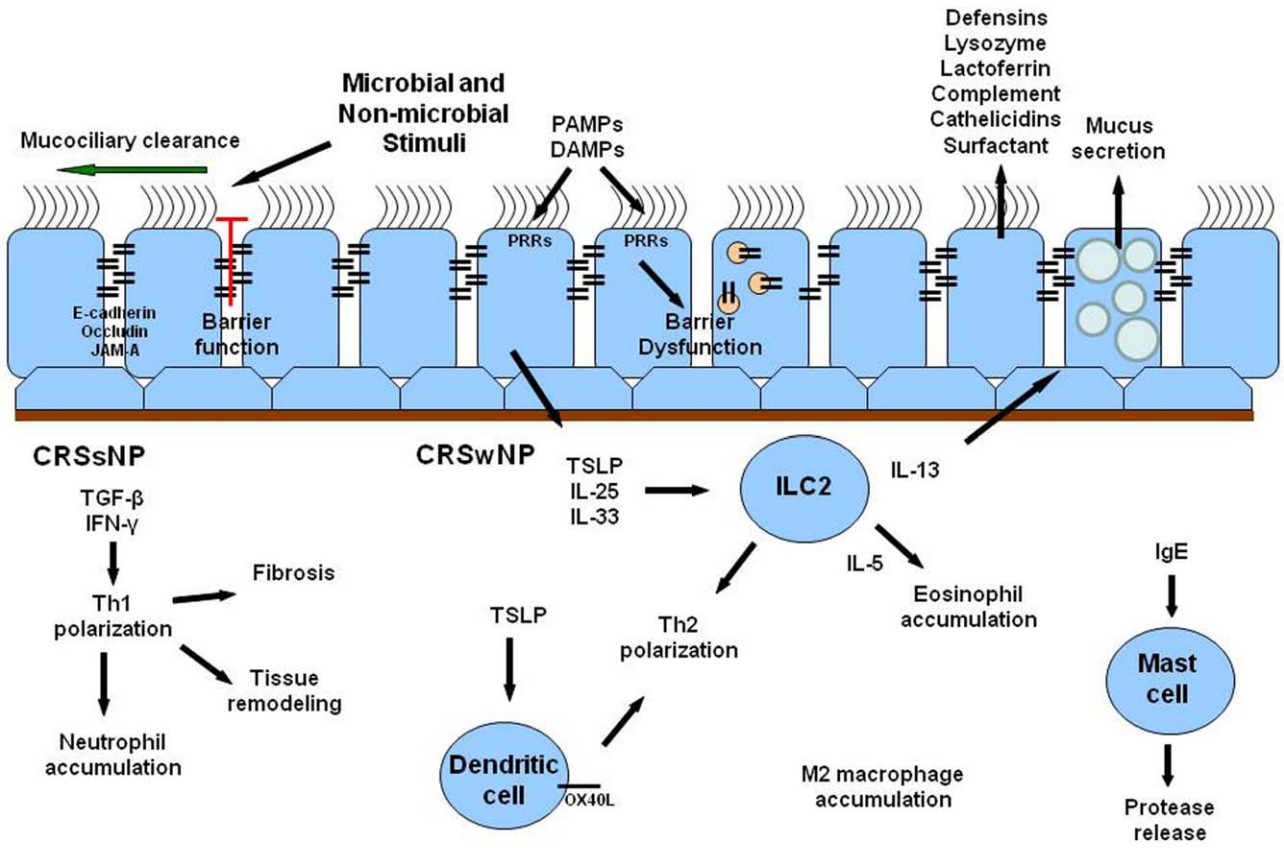

Fig. 1. Interplay between innate immune mechanisms may contribute to CRS pathophysiology. Microbial stimuli such as bacteria, viruses, and parasites and nonmicrobial stimuli such as allergens, particulate matter, and toxins are combated by mucociliary clearance and sinonasal epithelial barrier function to prevent exposure to the underlying tissue. PAMPs and DAMPs acting through PRRs such as extraoral taste receptors may activate an immediate and rapid counter response, whereas additional PRR downstream signaling through TLRs may activate other and likely redundant mechanisms, resulting in inflammatory cytokine release. In the case of CRSsNP, TGF-β, and IFN-γ release contribute to Th1 polarization, fibrosis, tissue remodeling, and neutrophil accumulation. In the case of CRSwNP, TSLP, IL-25, and IL-33 act on ILC2 and dendritic cells to promote a Th2 T and B cell response. Subsequent IL-5 release results in eosinophil accumulation and IgE induces protease release from mast cells. M2 macrophages are also recruited in CRSwNP. IL-13 release further stimulates secretion of mucus, surfactant, and antimicrobial peptides such as human beta defensins, acidic mammalian chitinase, lysozyme, lactoferrin, complement components, and cathelicidins.
CRS = chronic rhinosinusitis; CRSsNP = CRS without nasal polyps; CRSwNP = CRS with nasal polyps; DAMP = damage-associated molecular patterns; IFN-γ = interferon-γ; IL- = interleukin; ILC = innate lymphoid cells; PAMP = pathogen-associated molecular patterns; PRR = pattern recognition receptors; TGF-β = transforming growth factor beta; Th = T-helper; TLR = toll-like receptors; TSLP = thymic stromal lymphopoietin.

developed polyps in the lateral sinuses and/or the maxillary sinuses, none of the mast cell-deficient mice demonstrated polyps.[68] These polyp-like structures were characterized by an undulated basement membrane, cystic changes, and eosinophil accumulation in the parenchyma or lamina propria. Using MicroCT scan, this group also demonstrated that the mucosal thickening seen in wild-type mice treated with OVA was significantly decreased in mast cell-deficient mice. Goblet cell hyperplasia and epithelial cells hypertrophy was also significantly improved in mast cell-deficient mice. Thus these data demonstrate that mast cells contribute to multiple phenotypes of CRS in a mouse model of CRS.

Innate lymphoid cells are of lymphoid lineage, produce inflammatory cytokines, and may contribute to CRS.[59,69] They are divided into ILC1, ILC2, and ILC3 depending on the inflammatory cytokines and transcription factors they express.[59] ILC2s have been particularly implicated in CRSwNP because they are a major source of IL-4, IL-5, and IL-13, in addition to Th2 cells. GATA3- and retinoic acid receptor-related orphan receptor alpha are necessary for ILC2 development.[69] Innate lymphoid cells are activated by cytokines derived from epithelial cells, including IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), as well as lipid mediators.[69] ILC2s are significantly enriched in eosinophilic versus noneosinophilic nasal polyps, suggesting a role in CRSwNP pathogenesis.[70] Studies of the role of ILCs in sinonasal inflammation in animal models has been limited by difficulty in obtaining ILC2s in sinus mucosa. However, ILC2s in allergen-induced eosinophilia in the lung has been investigated.[70] Many of the studies of ILC2 in the mouse lung have harnessed RAG knockout mice; these lack B and T cells but retain ILC2s.[69] Depletion of ILC2s with anti-Thy1.2 antibody results in reduced airway hypersensitivity after influenza infection.[71] ILC2s have also been reported to contribute to allergen-induced lung inflammation including OVA, fungal allergen, and house dust mite.[69]

Network of Intercellular Signaling

In addition to singular roles and contributions detailed above of sinonasal epithelial cells, dendritic cells, mast cells, and ILC2s, these cells types form a complex network of stimulatory signals that interact on each other as well as peripheral blood leukocytes and cells from the adaptive immune system. The sinonasal epithelium is at the center of this signaling network (Fig. 1). In response to PAMPs and upon PRR activation, multiple cytokines are secreted, including TSLP, IL-25, and IL-33.[72,73] These cytokines have been demonstrated to be sufficient to generate Th2 responses and Th2 inflammatory cytokine profiles.[74,75] Furthermore, TSLP, IL-25, and IL-33 act upon multiple cell types, including ILC2s.[76] In response to these cytokines, ILC2s in turn secrete IL-13, which acts upon the epithelium to induce mucous secretion, as well as IL-5, which recruits eosinophil accumulation.[76]

As previously discussed, myeloid dendritic cells have been reported by several groups to be increased in CRSwNP.[77] These cells activate and polarize T-helper cells through stimulatory molecules such as OX40 ligand (OX40L) and programmed death ligand 1. Indeed, the percentage of dendritic cells expressing these ligands has been demonstrated to be increased in dendritic cells from patients with eosinophilic CRSwNP compared to controls and noneosinophilic CRSwNP.[77] In return, T cells via ligands such as CD40 ligand (CD40L) stimulate dendritic cells to secrete cytokines, including IL-6, IL-12, and transforming growth factor beta. Interestingly, dendritic cells isolated from sinonasal mucosal tissue from CRS patients demonstrated increased secretion of these cytokines compared to controls upon CD40L stimulation.[77] Further adding to the complex interplay is evidence that dendritic cells are activated to express ligands such as OX40L in response to TSLP.[59,78] As TSLP is known to be strongly expressed by the sinonasal epithelium, epithelial activation may therefore prime dendritic cells to express OX40L and lead to Th2 polarization and a dysregulated cycle of cytokine secretion and inflammatory cell activation.

CONCLUSION

Chronic rhinosinusitis is a heterogeneous multifactorial disease characterized by dysregulated inflammation. Abnormalities in innate immune function, including epithelial barrier integrity, mucociliary clearance, response to PAMPs via PRRs, and the contribution of innate immune cells through independent or interconnected signaling networks, may contribute to CRS pathophysiology (Fig. 1). Animal models with genetic deletions or depletion of innate immune cells, as presented in this review, are powerful tools that have elucidated the importance of these mechanisms, particularly in allergic inflammation (Table I). Whereas animal models have a clear and critical role in advancing scientific understanding of underlying cellular and molecular pathways in upper airway inflammation, key limitations prevent drawing direct conclusions about the human disease from experimental results in animals. Continued development of novel animal model systems will be necessary to further advance bench-to-bedside research in the field.

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