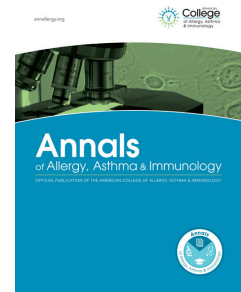


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Tuft cells in the pathogenesis of chronic rhinosinusitis with nasal polyps and asthma

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Running head: Tuft cells in Nasal Polyp and Asthma Pathogenesis

Keywords: asthma; chronic rhinosinusitis; nasal polyps; solitary chemosensory cell; brush cell; microvillus cell; tuft cell

Abbreviations/acronyms: chronic rhinosinusitis (CRS); chronic rhinosinusitis with nasal polyps (CRSwNP); chronic sinusitis without nasal polyps (CRSsNP); Center for Disease Control (CDC); microvillus cells (MVCs); solitary chemosensory cells (SCCs); neuroendocrine cells (NECs); calcitonin gene-related peptide (CGRP); γ -aminobutyric acid (GABA); innate lymphoid group 2 cells (ILC2s); cystic fibrosis transmembrane conductance regulator (CFTR); sweet and umami taste transduction system (T1R); sweet taste transduction system (T2R or TAS2Rs); phospholipase C β 2 (PLC β 2); cation channel transient receptor potential melastatin 5 (TRPM5);

succinate receptor 1 (SUCNR1); acetylcholine (ACh); T-helper type 1 inflammatory profile (Th1); thymic stromal lymphopoietin (TSLP); bronchoalveolar lavage (BAL); dendritic cell (DC); cysteinyl leukotrienes (CysLTs); leukotriene E₄ (LTE₄); leukotriene A₄ (LTA₄); 5-lipoxygenase (5-LO); 5-LO-activating-protein (FLAP); leukotriene B₄ (LTB₄); classical type 1 and 2 CysLT receptors (CysLT₁R and CysLT₂R); CysLT receptor 3 (CysLT₃R or GPR99 or OXGR1); gastrointestinal (GI); genitourinary (GU); house dust mite (HDM); C-X-C motif chemokine ligand 12 (CXCL12); Doublecortin-like Kinase (DCLK1/DCAMKL1); dextran sodium sulfate (DSS); Substance P (SubP); interleukin (IL); chronic obstructive pulmonary disease (COPD); cystic fibrosis (CF)

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Tables: 1

Conflict of interest: None

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Objective: To review the latest discoveries regarding the role of tuft cells in the pathogenesis of chronic rhinosinusitis with nasal polyposis and asthma.

Data Sources: Reviews and primary research manuscripts were identified from PubMed, Google and Bioarchives using the search words airway epithelium, nasal polyposis, chronic rhinosinusitis, or asthma AND chemoreceptor cell, solitary chemosensory cell, brush cell, microvillus cell, or tuft cell.

Study Selections: Studies were selected on the basis of novelty and likely relevance to the functions of tuft cells in chronic inflammatory diseases in the upper and lower airways.

Results: Tuft cells coordinate a variety of immune responses throughout the body. Following the activation of bitter taste receptors, tuft cells coordinate the secretion of antimicrobial products by adjacent epithelial cells and initiate the calcium dependent release of acetylcholine resulting in neurogenic inflammation, including mast cell degranulation and plasma extravasation. Tuft cells are also the dominant source of IL25 and a significant source of CysLTs that play a role in initiating inflammatory processes in the airway. Tuft cells have also been shown to appear de novo in the distal airway following viral infection, implicating these cells in dysplastic remodeling in the distal lung in the pathogenesis of asthma.

Conclusion: Tuft cells bridge innate and adaptive immune responses and play an upstream role in initiating Type 2 inflammation in the upper and possibly the lower airway. The role of tuft cells in respiratory pathophysiology must be further investigated as tuft cells are putative high value therapeutic targets for novel therapeutics in CRSwNP and asthma.

1 Introduction

2 Inflammatory diseases of the respiratory system—including chronic rhinosinusitis (CRS)
3 and asthma affecting the upper and lower airway, respectively—have a major negative impact
4 on the human population. The Center for Disease Control (CDC) reports a 11.2% rhinosinusitis
5 prevalence and a 7.6% asthma prevalence in the United States,¹ which account for billions of
6 dollars in healthcare spending annually including almost 25% of annual adult antibiotic
7 prescriptions.^{2,3,4} Within the last decade, it has been increasingly acknowledged that both CRS
8 and asthma are heterogenous diseases characterized by a spectrum of inflammatory profiles
9 that encompass distinct clinical presentations.⁵ Although Type 2 inflammation often underlies
10 CRS and asthma pathogenesis, recent genotypic and molecular studies have revealed
11 important new insights into the diverse cellular mechanisms driving these two clinical conditions.
12 A broad spectrum of inflammatory profiles and endotypes is now appreciated in CRS patients
13 with and without nasal polyposis.⁶ Specifically, the role of rare epithelial cell types, especially tuft
14 cells which are members of the chemosensory family and include brush cells, microvillus cells
15 (MVCs) and solitary chemosensory cells (SCCs), has recently been uncovered. This review will
16 focus on what is currently known regarding the functions of tuft cells in the airway, with specific
17 regard to how tuft cells functionally bridge innate and adaptive responses that underlie Type 2
18 inflammatory disease pathophysiology.

19 Rare Cell Types in the Respiratory Tract

20 The respiratory tract filters approximately 10,000 liters of air each day and functions as
21 an important barrier serving as a first line of defense against inspired airborne pathogens.⁷ A
22 pseudostratified epithelium functions to initiate both innate and adaptive immune responses and
23 undergoes complex tissue regenerative processes that mediate tissue repair following infectious
24 and non-infectious injury.⁸ Basal progenitor cells, ciliated cells and secretory cells comprise the
25 majority of the respiratory epithelium, but in addition there are at least three rare epithelial
26 subtypes that mediate important homeostatic functions throughout the respiratory tract (Table

1). Neuroendocrine cells (NECs) are neurosensory cells dispersed throughout the respiratory epithelium, often in clusters of 20-30 cells near airway branch points, with the capacity to act as reserve stem cells during lung injury.⁹ NECs are increased in both number and cluster size in humans with a variety of lung diseases including asthma.¹⁰ NECs have been shown in non-human models to secrete neuropeptides including calcitonin gene-related peptide (CGRP) and γ -aminobutyric acid (GABA), modulating the production of inflammatory cytokines by innate lymphoid group 2 cells (ILC2s)¹¹ and inducing goblet cell hyperplasia.¹² Ionocytes are rare cells that are greatly enriched in ion transporters and are the major source of transcripts of the cystic fibrosis transmembrane conductance regulator (CFTR) in both the mouse and human airway.^{13,14} Ionocytes likely play an important role in controlling the epithelial barrier¹⁵ as well as the luminal pH in the airway,¹⁶ thereby regulating mucous viscosity. Lastly, members of the tuft cell family include brush cells, microvillus cells (MVCs) and solitary chemosensory cells (SCCs), use taste transduction components to survey the extracellular environment and can initiate both innate and adaptive immune responses in the airway. Mouse and human studies demonstrate that these cells play an upstream role in the initiation of Type 2 inflammation by stimulating neurogenic inflammation and generating IL-25¹⁷ and CysLTs.¹⁸

Characterizing Tuft cells of the Airway

Tuft cells have been characterized in a wide variety of anatomical locations in humans including the nasal cavity (both the respiratory and olfactory epithelia of the nose), pharynx, larynx, trachea, proximal airway, intestine (including the gastric mucosa as well as the small and large intestine and the pancreato-biliary system), urethra as well as the murine thymic medulla and the auditory canals and nasal aspects of the conjunctiva.^{19,20,21} Tuft cells are termed SCCs in the sinonasal and lung epithelia,²² brush cells in the trachea²³ and tuft cells in the GU and intestinal tract,^{24,25,26} named for their distinctive microvilli that form a “tuft” extending into the apical lumen. SCCs are conserved within the nasal epithelium of rodents, humans and reptiles.²⁷ Microvillus cells (MVCs) are tuft cells present in the human olfactory epithelium²⁸ that

share morphologic features with SCCs. SCCs express components of the sweet and umami (T1R) taste transduction system as well as the bitter (T2R) taste transduction system, although T1Rs are expressed at a much lower level than T2Rs.²⁰ The T2R system utilizes a low-affinity G-protein coupled to 7 transmembrane spanning domain receptors for bitter stimuli known as T2Rs (TAS2Rs). The trimeric G-protein, named gustducin²⁹ in humans consists of the α -subunit encoded by GNAT3³⁰ and a $\beta\gamma$ dimer. When dissociated by activation of the T2R, gustducin activates phosphodiesterase reducing intracellular cAMP³¹ while the $\beta\gamma$ dimer couples to downstream effectors including phospholipase C β 2 (PLC β 2), which results in 1,4,5-inositol trisphosphate mediated release of Ca²⁺ resulting in activation of the cation channel transient receptor potential melastatin 5 (TRPM5).^{32,33} The transcription factor *Pou2f3* that specifies the tuft cell lineage,²⁴ DCAMKL-1³⁴ and others make up the reproducible mRNA transcriptional signature common to tuft cells.³⁵ Although recognized as members of the same chemosensory family, regional variation in receptor expression is seen among tuft cells. SCCs, tracheal brush cells and chemosensory cells of the thymus express receptors for the bitter taste transduction system at higher levels than intestinal tuft cells,^{35,36} while brush cells and intestinal tuft cells have been shown to express succinate receptor 1 (SUCNR1), which recognizes succinate and has been shown to be important in the Type 2 inflammatory response in helminth and protozoal infections.^{37,38} Additionally, both MVCs and SCCs express high levels of transcripts for production of IL-25 and the CysLT biosynthetic pathway.^{39,18}

Tuft cells Coordinate Secretion of Antimicrobial Products and Facilitate Host Defense

Epithelial cells luminally secrete a broad collection of antimicrobial products that help promote homeostasis in the airway. Epithelial cells produce these compounds following activation of an array of pattern recognition receptors including C-type lectin receptors, Nod-like receptors, Rig-like receptors and Toll-like receptors, although an overview of these receptors and pathways is beyond the scope of this review. Tuft cells are involved in these antimicrobial

defenses and stimulate the secretion of antimicrobial products from adjacent epithelial cells following SCC bitter taste receptor activation (Figure 1). Bitter and sweet taste receptors recognize a variety of bacterial products and play an important role in the innate immune response throughout the human body.⁴⁰ When tuft cells in the upper airway are stimulated by T2R bitter taste receptor agonists, such as denatonium benzoate, parthenolide and amarogentin, an intracellular calcium wave is generated that propagates to surrounding epithelial cells via gap junctions resulting in the release of antimicrobial peptides such as β -defensins-1 and -2.⁴¹ Additionally, activation of sweet receptors (T1Rs) suppresses antimicrobial peptide secretion induced by T2Rs in response to bitter agonists and presumed bacterial products. Interestingly, patients with persistent sinonasal inflammation (CRS) have elevated levels of glucose in their nasal secretions capable of blunting the SCC-mediated antimicrobial peptide release.⁴¹

There are 25 T2Rs in humans and nearly 90 common (minor allele frequency ≥ 0.05) functional polymorphisms in these receptor genes, which could underlie mechanisms of both taste preferences for certain foods and differences in immune mediated pathogen clearance from the airway in different individuals. In particular, these mutations could underlie hereditary and genetic predispositions noted in some cohorts of CRS patients (see below).^{43,43} Certain variants in the gene that encodes for T2R38, a receptor expressed on sinonasal ciliated cells that stimulates local nitric oxide production, are associated with an increased susceptibility to infections of the upper airway in CRSsNP⁴⁴ and these polymorphisms in T2R38 appear to predict surgical outcomes for patients with CRSsNP.^{45,46,47} Recently, T2R38 has been shown to be expressed not only on ciliated epithelial cells, but also on cells of the adaptive immune system including myeloid cells,⁴⁸ lymphocytes⁴⁹ and macrophages in the lung,⁵⁰ although the implications for this expression in the context of antimicrobial activity and Type 2 inflammatory disorders remain unclear. While the role of the bitter taste receptor T2R38 in ciliated cell innate immunity has been described, the role of taste receptors in tuft cells is currently under rigorous

investigation, and it is becoming increasingly clear that these cells coordinate immune responses throughout the body.

Acetylcholine Released by Tuft Cells is Implicated in the Pathogenesis of Pulmonary Diseases

Early morphological studies noted that tuft cells in the upper airways were densely supplied with nerve endings on their basolateral surface that were noted to be immunoreactive for calcitonin gene-related peptide (CGRP) and substance P.⁴⁸ It was later found that murine brush cells in the trachea express markers not only of the bitter taste transduction system (T2Rs, PLC β 2, α -gustducin) but are also connected to cholinergic sensory nerve fibers. Upon stimulation, murine tuft cells in the trachea have been shown to release acetylcholine, resulting in an abrupt decrease in respiratory rate and stimulation of respiratory reflexes.⁵¹ The expression of choline acetyltransferase (ChAT) can be detected in most tuft cells, including human tuft cells in the respiratory tract,⁵² and a wide variety of signals including ATP, bitter compounds, cigarette smoke and bacterial metabolites are known to trigger the release of acetylcholine from tuft cells.⁵³ Murine nasal tuft cells are activated by the bitter taste receptor agonist denatonium benzoate as well as acyl-homoserine lactones, which are quorum-sensing molecules secreted by gram-negative bacteria including *Pseudomonas aeruginosa*, resulting in an apneic reflex⁵⁴ as well as stimulation of trigeminal nociceptive nerve fibers and local neurogenic inflammation, including mast cell degranulation and plasma leakage.⁵⁵

The production of acetylcholine by tuft cells has been implicated in a variety of biological processes in the upper and lower airway, including maintaining epithelial homeostasis⁵⁶ and mucociliary clearance.⁵⁷ A well-accepted vasoconstrictor of smooth muscle cells in the airway, acetylcholine is traditionally thought to exert its effects following release from cholinergic nerve terminals; however, acetylcholine derived from tuft cells and non-neuronal sources has recently been implicated in the regulation of airway smooth muscle tone.⁵⁸ Although bronchiolar hyperresponsiveness and allergic inflammation are inhibited by vagotomy of parasympathetic

130 nerves in the lungs in a canine model,⁵⁹ suggesting that neuronal acetylcholine plays an
 131 essential role in the pathogenesis of asthma, acetylcholine derived from tuft cells is still thought
 132 to play a role in the pathogenesis of pulmonary diseases as well as Hirschsprung's disease⁶⁰
 133 and several cancers.^{61,62} Given that cholinergic bronchoconstriction is an important clinical
 134 feature of asthma, tuft cells and innate immune mechanisms of acetylcholine production could
 135 provide a new target for novel therapeutic interventions. Despite the importance of these
 136 findings with regard to human disease, the details of the mechanisms by which acetylcholine is
 137 synthesized and released by tuft cells still remain largely unknown.

138 **Pathogenesis of Type 2 Inflammation in CRSwNP in the Upper Airway and Asthma in the** 139 **Lower Airway**

140 Chronic rhinosinusitis (CRS) is classically differentiated into two types, with and without
 141 nasal polyps (CRSwNP and CRSsNP, respectively). Historically, CRSsNP was thought to be
 142 mediated at a cellular level by T-helper type 1 (Th1) inflammatory profile (interferon gamma)
 143 along with the Th3 inflammatory profile (IL-17) and CRSwNP by a Type 2 inflammatory profile
 144 (IL-4, IL-5, IL-13); however recently CRS has increasingly been recognized as a spectrum of
 145 inflammatory processes (Figure 2). Although CRSwNP is found most often to possess a Type 2
 146 inflammatory profile, CRSsNP can also bear a Type 2 inflammatory profile or an entirely
 147 different profile⁶³, while CRSwNP can have a significant Th1 contribution.⁶⁴ A notable form
 148 CRSwNP is characterized by high levels of cytokines IL-4, IL-5 and IL-13 as well as high
 149 concentrations of eosinophils, ILC2s and mast cells and is often referred to as eosinophilic Type
 150 2 inflammation.⁶⁵ High concentrations of epithelial cytokines IL-25, thymic stromal lymphopoietin
 151 (TSLP) and IL-33 have also been reported in patients with eosinophilic Type 2 inflammation with
 152 CRSwNP⁶⁶ and asthma.⁶⁷ Although experiments in mice and humans demonstrated that these
 153 cytokines are required for Type 2 immunity in the intestine, the sources and regulation of these
 154 cytokines remained unclear until recently. In murine studies, IL-25-deficient mice exhibited a
 155 substantial suppression of proinflammatory mediators and eosinophils in bronchoalveolar

lavage (BAL) fluids as well as decreased IgE levels upon induction of Type 2 inflammation. IL-25 has been reported to enhance the differentiation of naïve T cells into Th2 cells, both directly⁶⁸ and indirectly⁶⁹ by modulating dendritic cell (DC) function. The IL-25 deficient mice were noted to have unaffected dendritic cell (DC) migration and antigen-specific Type 2 cell expansion, suggesting that IL-25 is essential for the activation of Th2 cells in the elicitation phase but not induction of antigen-specific Th2 differentiation in the sensitization phase of Type 2 inflammation in the airway. In a mouse model for asthma, IL-25 derived from the epithelium and distinct from IL-25 produced by hematopoietic cells was required for the production of IL-13 during Type 2 inflammation. IL-25 produced by activated epithelial cells in the airway is required in and responsible for initiation of eosinophilia in Type 2 inflammation, but not for Type 2 cell differentiation in a murine model for asthma,⁷⁰ and these results have been reproduced in human studies implicating IL-25 and tuft cells in Type 2 inflammatory diseases.

Tuft cells are the Dominant Source of IL-25, Key Cytokine in Initiation of Type 2 Inflammation

Tuft cells are known to constitutively secrete IL-25, which acts to sustain ILC2 homeostasis in the murine intestinal epithelium. Upon infection by helminths and in response to IL-4R activation²⁴ and activation of the succinate receptor *Sucnr1*,³⁷ tuft cells increase production of IL-25, which triggers production of IL-13 by ILC2s ultimately resulting in increased frequencies of tuft and goblet cells.^{26,38} Like in the intestinal epithelium, tuft cells in the human sinonasal epithelium also produce IL-25, which is apically secreted by human sinonasal epithelial cultures following IL-13 treatment. Consistent with this in vitro observation, patients with CRSwNP have higher levels of IL-25 in their mucus secretions compared to patients with CRSSNP,¹⁷ which is consistent with the theory that prolonged exposure to IL-13 results in proliferation of tuft cells resulting in more IL-25 and a “leaky” epithelium, allowing IL-25 to translocate from the apical surface to the basolateral environment and act on ILC2 cells. Investigating the IL-25 levels of patients with CRSwNP could be used as an important biomarker

in guiding treatment for patients with Type 2 inflammatory diseases. Additionally, anti-IL-25 or anti-IL-13 monoclonal antibodies should be investigated as potential therapies for treating patients with CRS in the pre- and postoperative settings.

Tuft Cells are Enriched in Human Nasal Polyp Tissue

Analysis of human sinonasal single-cell RNAseq data has demonstrated that tuft cell markers are expressed at higher levels in the sinus than in the nasal cavity in patients with CRS, implicating tuft cells in the pathogenesis of CRSwNP.⁷¹ It was further demonstrated that SCCs and brush cells proliferate upon exposure to aeroallergens including fungal extracts (*Aspergillus fumigatus* and *Alternaria alternata*)^{39,36} as well as following H1N1 influenza virus infection.⁷² Recently, a gustducin/DCAMKL1-positive cell population was identified that is increased in human polyp tissue compared with turbinate tissue. The group reported that sinonasal polyp derived cultures from both submerged epithelia (non-differentiated) as well as cultures with biphasic epithelia (air liquid interface) produced IL-25 following stimulation with IL-13, possibly suggesting that differentiation does not need to occur completely in order for IL-25 production to occur. Indeed, populations of DCAMKL1-positive cells are increased in human serrated colorectal polyps⁷³ as well as non-small cell lung cancer⁷⁴ and have been studied for their prospective role in epithelial-to-mesenchymal transition.⁷⁵ Together, high concentrations of tuft cells and/or tuft cell-progenitor cells coupled with persistent epithelial barrier perturbation may be important roles in the origination of Type 2 inflammation in the upper airway.

Tuft Cell-Derived Eicosanoids Contribute to Type 2 Inflammation

Cysteinyl Leukotrienes (CysLTs) are a subclass of eicosanoids that act as inflammatory mediators. Eicosanoids including CysLT leukotriene E₄ (LTE₄) are stable metabolites that have long been known to be increased in tracheal aspirates^{76,77} and urine⁷⁸ of patients with asthma exacerbations. LTE₄ has been shown to cause airway constriction, plasma leakage and eosinophil accumulation in the bronchial mucosa in humans.⁷⁷ Initial investigations into the biosynthesis of LTE₄ revealed that arachidonic acid is converted in a two-step pathway to

leukotriene A₄ (LTA₄) by 5-lipoxygenase (5-LO), during which Ca²⁺ targets 5-LO to the nuclear membrane⁷⁹ where LTA₄ is then transformed to LTC₄, exported and converted extracellularly to LTD₄ and LTE₄. In response to fungal antigens, mast cells are capable of producing high levels of leukotriene B₄ (LTB₄) and LTC₄ using the co-receptor dectin-1, a Syk tyrosine kinase-dependent pathway,⁸⁰ and murine bone marrow-derived dendritic cells are capable of producing CysLTs through dectin-2 recognition.⁸¹ In addition, intestinal tuft cells,⁸² tracheal brush cells³⁶ and sinonasal and lung SCCs¹⁴ are known to express high levels of transcripts for CysLT biosynthetic enzymes. In the small intestine, tuft cells are known to regulate the Type 2 immune response to helminths through the secretion of IL-25 and tuft-cell derived leukotrienes.⁸³

Although LTE₄ was found to bind much more weakly to the classical type 1 and 2 CysLT receptors (CysLT₁R and CysLT₂R) than LTC₄ or LTD₄, it was found that LTE₄ produced hypersensitivity reactions in the airways of patients with asthma,⁷⁷ prompting investigation into novel receptors that might be selective for LTE₄ in the context of Type 2 inflammation. CysLT receptor 3 (CysLT₃R) (GPR99 or OXGR1) was identified to have a high affinity for LTE₄ and mediate vascular permeability and mucin release.⁸⁴ Unlike CysLT₁R and CysLT₂R, CysLT₃R is thought to be predominantly an epithelial receptor, thus implicating not only high concentrations of leukotrienes but also the expression of CysLT₃R on neighboring epithelial cells in the pathophysiology of Type 2 inflammation.⁸⁵ In mouse models, CysLT₃R expressed on epithelial cells sense LTE₄ and alone can drive IL-25-dependent proliferation of brush cells in the trachea.³⁶ Additionally, aeroallergens and ATP results in brush cell production of CysLTs through P2Y2 and P2X4 receptor activation.¹⁸ Brush cell deficient *Pou2f3*^{-/-} mice produce significantly less CysLTs upon exposure to mold aeroallergen *Alternaria* and the house dust mite *Dp*,¹⁸ indicating that tuft cells are a significant source of CysLTs in the airway that contribute to Type 2 inflammation.

In addition to CysLTs, tuft cells have also been shown to produce prostaglandin D₂ (PGD₂) in a mouse model.⁸⁶ Tuft cells are known to express ALOX5, COX1, COX2 as well as

hematopoietic prostaglandin D synthase,¹³ perhaps as part of the constitutive activity of these cells within the epithelial barrier. Although tuft cells lack the enzymes required for conversion of PGD₂ to PGE₂, the effect of tuft-cell derived PGD₂ on neighboring epithelial cell types has yet to be fully explored. Additionally, both ILC2s and Th2 cells express CRTH2,⁸⁷ a PGD₂ receptor, in line with the success of Fevipiprant, a PGD₂ receptor antagonist, in clinical trials.⁸⁸ The effect of prostaglandins on tuft cells themselves is another area warranting further investigation. It has also been shown that tuft cell frequency decreases in response to increased levels of PGE₂ after observing reduced numbers of tuft cells in the colon with decreased myeloid phagocytic activity.⁸⁹ The role of feedback and the effect of prostaglandins on tuft cells has yet to be fully explored, and could illuminate the details of the mechanisms underlying asthma as well as inspire novel therapeutic approaches.

Aspirin-exacerbated respiratory disease (AERD) is a distinct chronic pro-inflammatory condition characterized by CRSwNP, asthma, and sensitivity to medications that inhibit cyclooxygenase-1 (COX-1) enzymes. Similar to CRSwNP, a hallmark of AERD is Th2 inflammation with increased IL-25 levels; however, levels of eosinophil and mast cell degranulation and disease severity are greater in AERD.^{90,91,92,93} In addition, AERD, unlike CRSwNP, also involves baseline PGE₂ deficiency and increased 5-lipoxygenase activity that is amplified by COX-1 inhibition (e.g., by aspirin)^{94,95} suggesting a possible role of tuft cell biology in the disease process. As mentioned above, both human and mouse nasal tuft cells utilize bitter taste receptor signaling and are activated by the bitter tasting molecule denatonium benzoate.^{23,41,51,54} Furthermore, bitter taste receptor function is genetically variable among people governing both bitter taste perception as well as innate defense functions.^{44,45,46,47} Prior work demonstrated that cellular hyperresponsiveness to denatonium benzoate predicts worse surgical outcomes in CRSwNP patients⁹⁶ and AERD patients report increased bitter taste perception to denatonium compared to non-AERD individuals.⁹⁷ Thus, it is suggested that

genetic differences in bitter taste receptors expressed on human nasal tuft cells may be predisposing factors to developing AERD.

Tuft Cells are a Central Feature of Dysplastic Remodeling in the Distal Lung

Recently, the *de novo* appearance of tuft cells in the lower airway, specifically the distal mouse lung, has been observed following infection by a H1N1 influenza virus.⁷² H1N1 influenza virus infection resulted in widespread dysplastic alveolar remodeling that included development of tuft cells in the distal lung. Subsequent activation of these tuft cells by stimulation with bitter taste receptor agonists and succinate triggered vasodilation and plasma leakage, potentially explaining how childhood viral infections can predispose patients to develop asthma. However, it was still unclear whether tuft cells in humans cause vascular permeability through neurogenic inflammation via acetylcholine release or whether Type 2 cytokines produced by tuft cells indirectly cause vascular permeability and plasma leakage. Regardless, it is evident that tuft cells function as mediators of inflammation and dysplasia, which has important consequences for understanding CRSwNP and asthma pathogenesis.

Conclusion

While the role of tuft cell lineages in promoting gastrointestinal (GI) Type 2 responses is well-established²¹⁻²³, their role in shaping respiratory pathophysiology is less well characterized. In chronic airway diseases such as CRSwNP and allergic asthma, further investigation of how airway tuft cells use gustatory signaling cascades to regulate production of IL-25, CysLTs, and acetylcholine is necessary. Here we propose new avenues for investigating respiratory tuft cell heterogeneity and for uncovering aspects of their biology that extend beyond Type 2 responses.

Recently, the ATP-sensing purigenic receptor P2Y2 has been shown to play a role in promoting CysLT production from olfactory nasal brush cells, demonstrating a striking diversity in GPCR transcripts within these cells¹⁷. This creates ample opportunity to uncover novel signals operating upstream of GPCR signaling that mediate tuft cell activation³¹. It is currently thought that all tuft cell signaling cascades propagate through the Ca²⁺-activated TRPM5

monovalent cation channel implying that ligand binding to as yet undefined GPCRs could co-opt taste signaling transduction components like α -gustducin to trigger depolarization-dependent release of tuft cell derived mediators. Whether complex allergenic components e.g. house dust mite (HDM) antigens could function as GPCR ligands for eliciting tuft cell mediator release needs to be tested. It was recently demonstrated that serum amyloid A1 is a soluble PRR that interacts with components of HDM to elicit IL-33 release from transformed and primary epithelial cells from CRS patients. Whether airway tuft cells express cell associated or soluble pattern recognition receptors to respond to inhaled antigens requires further study.

It is now clear that tracheal tuft cells cluster into three distinct populations: immature, tuft-1, and tuft-2¹². Type 1 tracheal tuft cells express higher levels of genes associated with taste transduction, while Type 2 tracheal tuft cells have increased expression of immune-related genes, notably mediators of leukotriene biosynthesis like *Alox5ap*¹². Nonetheless, both Type 1 and Type 2 tracheal tuft cells express high levels of *Tslp* and *IL25*¹², so it is not well understood whether heterogeneity of tuft cells in the trachea translates to different disease pathophysiologies. Similar heterogeneity has been described in small intestine tuft cells, where Type 1 tuft cells associated with patterns of neuron-associated genes whereas Type 2 shows patterns of innate immune genes, yet again, both populations express IL-25, its receptor IL-17RB and constituents of the IL-13 receptor (IL-4R α and IL-13R α 1).⁹⁹ Tuft cells expand in an IL-13-dependent fashion in human epithelial cell cultures from CRSwNP patients, so presumably the tuft cell-ILC2 circuit allows for expansion of tuft cells from surrounding basal cell progenitors.¹⁰⁰ It will be important to test whether chronic type 2 inflammatory diseases such as asthma and CRSwNP, basal cell differentiation is biased into particular tuft cell subsets that work in conjunction with distinct immune or neuronal cells populations. Employment of genetic tools for tracking Type 1 (*Pou2f3*) and Type 2 (*Gf1b*, *Spib*, and *Sox9*) tuft cell subsets will be useful to clarify their distinct roles in various respiratory diseases.

Regarding non-Type 2 inflammatory responses, tracheal brush cells express high levels of transcripts encoding IL-18, IL-10 and C-X-C motif chemokine ligand 12 (CXCL12)³⁸, which may be involved in host responses to microbial pathogens. While these studies need to be further explored, they suggest that airway tuft cells utilize immunomodulatory effector molecules outside of Type 2 polarizing cytokines. Also the role of the microtubule-associated protein Doublecortin-like Kinase (DCLK1/DCAMKL1) in tuft cells is unknown. While it is a common marker used to identify tuft cells across many tissues, DCLK1 is a hallmark of stem-like cells in cancer or after injury.¹⁰¹ Increased number of tuft cells have been observed during the initiation of tumorigenesis under inflammatory conditions in mouse models of gastric^{102,103} and pancreatic^{104,105} cancers. Notably, there is abundant *POU2F3* expression in small lung cell cancer variants that lack pulmonary neuroendocrine features, with high level expression of canonical tuft cell markers *TRPM5*, *GFI1B* and *CHAT*.⁵² Moreover, mice with an inducible *Dclk1*-driven tuft cell deficiency have poor epithelial recovery and survival in the dextran sodium sulfate (DSS) colitis model, suggesting a small population of intestinal DCLK1+ tuft cells may also be involved in tissue regeneration⁹⁰. While caveats in these models include DCLK1 not being exclusively expressed in tuft cells, they posit an interesting hypothesis where perhaps under chronic inflammatory conditions or activation of oncogenic mutations (APC, KRAS), the postmitotic status of tuft cells may be altered.

Better genetic tools and ways to manipulate the tuft cell lineage in mice, along with more precise descriptions of the distribution of tuft cell subsets in human airways are necessary to gain additional insights into the immunologic and non-immunologic roles for airway tuft cells, in a wide array of human chronic disease contexts.

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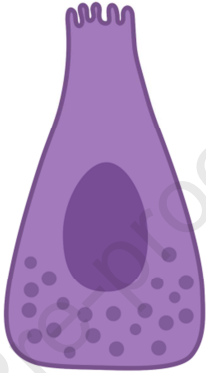
	<u>Tuft Cells</u>	<u>Pulmonary Neuroendocrine Cells (PNECs)</u>	<u>Ionocytes</u>
			
Location	Thinly distributed throughout the nasal cavity (both the respiratory and olfactory epithelia of the nose), pharynx, larynx, trachea, proximal airway, intestine (including the gastric mucosa as well as the small and large intestine and the pancreato-biliary system), murine thymic medulla, auditory canals and nasal aspects of the conjunctiva	Thinly distributed throughout the bronchial epithelium, often in innervated clusters of 20-30 at branch points	Thinly distributed throughout the bronchial epithelium, more concentrated in the bronchial gland ducts
Biology	Coordinate secretion of antimicrobial products, initiate neurogenic inflammation, secrete inflammatory mediators	Goblet cell hyperplasia, stimulate ILC2 cytokine production	Regulate epithelial surface mucous physiology including luminal pH
Secretes	ACh, SubP, CysLTs, IL-25	CGRP, GABA	
Notable channels expressed	TPRM5		CFTR
Neural Activation	yes	yes	no
Implicated in...	Asthma, CRS	Asthma, COPD, CF	CF

Table 1. Rare Epithelial Cell Types in the Respiratory Tract

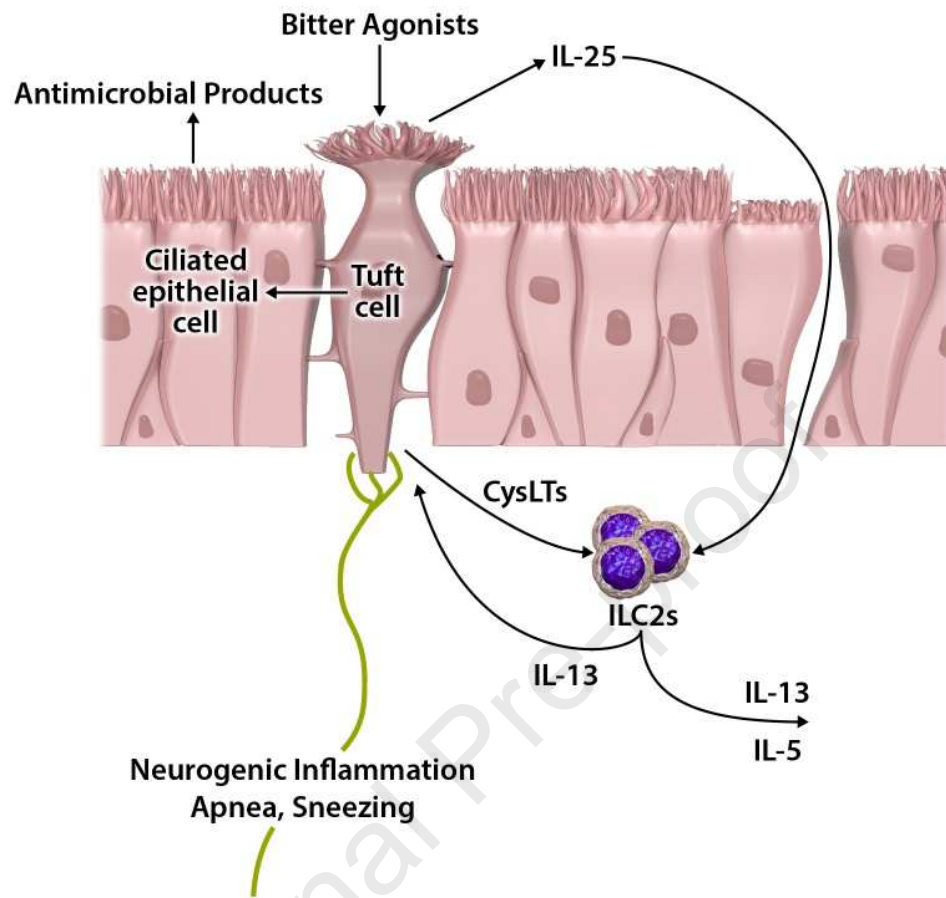


Figure 1. Tuft cells bridge innate and adaptive immune responses.

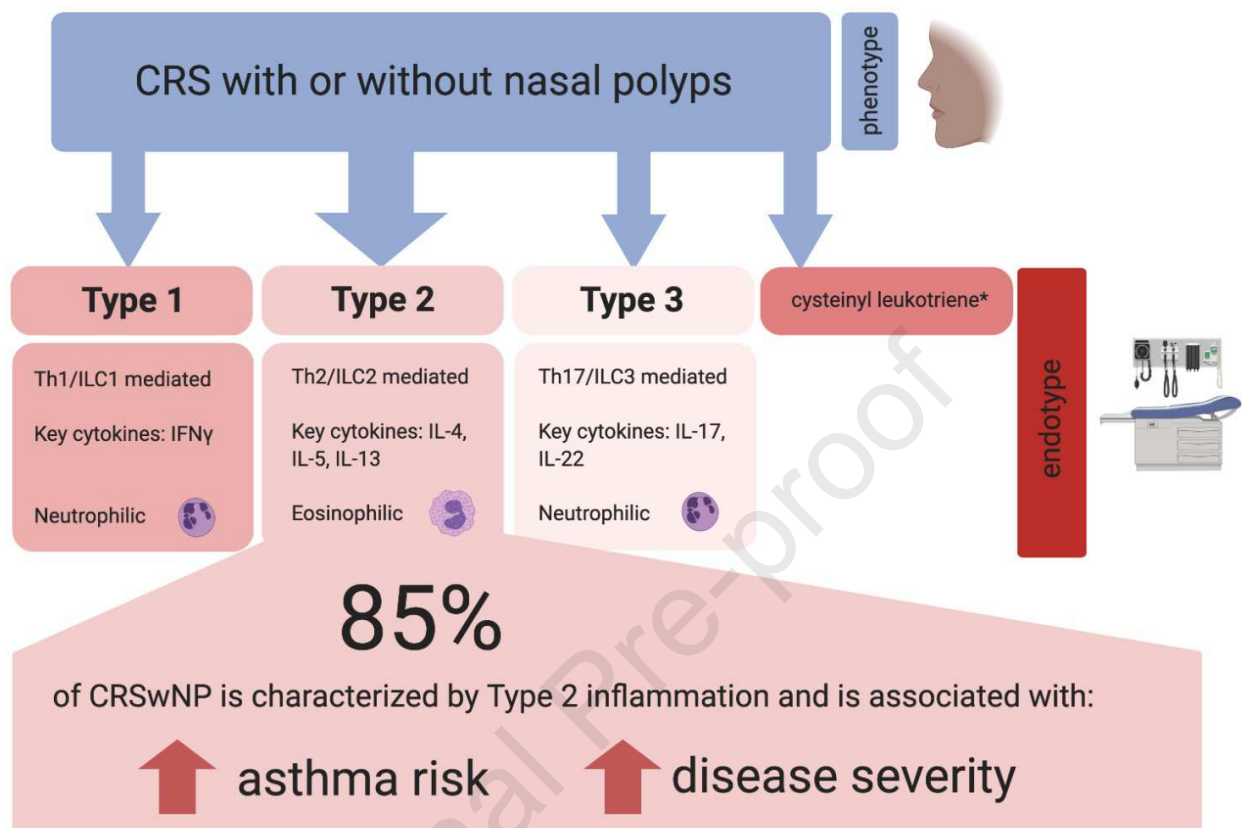


Figure 2. Phenotypes and Endotypes of CRS

Figure Legends

Figure 1. Upon stimulation by bitter agonists, tuft cells coordinate the release of antimicrobial peptides by neighboring ciliated epithelial cells. Tuft cells are also known to stimulate local inflammation through the release IL-25 and CysLTs that can stimulate ILC2 and downstream pathways leading to the release of IL-13, IL-5 and Type 2 inflammation. IL-13 also feeds back on tuft cells resulting in expansion. In the upper airway tuft cells are also intimately innervated by free nerve fibers of the trigeminal nerve and induce neurogenic inflammation through release of acetylcholine stimulating associated nerve fibers to release peptidergic neurotransmitters.

Figure 2. *Related to aspirin-exacerbated respiratory disease (AERD), a unique clinical phenotype of CRSwNP associated with both asthma and intolerance of cyclooxygenase-1 (COX-1) inhibiting agents.^{106,107,108,109} Created with [BioRender.com](https://www.biorender.com).

Table 1. Created with [BioRender.com](https://www.biorender.com).

Key Messages

- Tuft cells are a type of rare epithelial cell present in the respiratory and intestinal tracts among other locations in humans, rodents and reptiles.
- In addition to other functions, tuft cells use components of the taste transduction system to survey the extracellular environment and play a role in pathogen defense by stimulating neighboring ciliated epithelial cells to secrete antimicrobial products.
- The dominant epithelial source of IL-25 and a significant source of CysLTs in the airway, tuft cells have been implicated as upstream agents in Type 2 inflammation in CRSwNP and asthma.
- An important role of tuft cells is to act as a bridge between innate and adaptive immune responses in driving Type 2 inflammation in the airway.
- Tuft cells cluster into subpopulations, which could play diverse roles in different disease pathophysiologies and possibly utilize immunomodulatory effector molecules outside of Type 2 inflammatory cytokines.
- Further studies, particularly the use of better genetic tools for tracking tuft cells subpopulations, are needed to investigate the extent to which different subpopulations of tuft cells contribute to chronic inflammatory diseases.