Bitter Taste Receptors in Innate Immunity: T2R38 and Chronic Rhinosinusitis

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Abstract: Bitter taste receptors (T2Rs) serve a purpose far beyond taste sensation in the tongue; they have emerged as significant components of respiratory innate immune defense. T2R38, a specific T2R expressed in the airway, is activated by secreted products from gram-negative bacteria, and triggers nitric oxide (NO) production as a response. NO is directly bactericidal and also acts as a second messenger to increase ciliary beating and mucociliary clearance. T2R38 has common genetic polymorphisms that can render the receptor non-functional, and variations in functionality have demonstrated clinical impacts. Homozygotes for the non-functional form of the receptor have increased gram-negative bacterial proliferation *in vivo*, and these patients also are at a higher risk for chronic rhinosinusitis requiring functional endoscopic sinus surgery. Further studies have shown increased *in vitro* potential for biofilm formation in airway epithelial cells obtained from homozygote "non-taster" patients. Ongoing research into the clinical impact of T2R38 and other bitter taste receptors may yield novel therapeutics that leverage innate immune defense mechanisms and offer alternatives to conventional antibiotic treatment.

Keywords: T2R38, Bitter Taste Receptor, Chronic Rhinosinusitis, Sinonasal Immunity.

INTRODUCTION

Several immune mechanisms function in concert to maintain sterility of the sinonasal tract, a complex task due to its constant exposure to pathogens, debris, and environmental insults. Bitter taste receptors (T2Rs), once thought to be isolated to the tongue, have been identified in the airway and are critical components of innate immune defense [1-5]. These receptors sense the presence of bacterial products on the respiratory mucosal surface and then incite downstream antibacterial effects that can prevent chronic infection. T2R38, a specific bitter taste receptor with common genetic polymorphisms, is expressed in the nose and sinuses and is a key player in immunity, infection, and chronic rhinosinusitis (CRS).

INNATE AIRWAY IMMUNITY AND BITTER TASTE RECEPTORS

The surface of the nose and sinuses is almost entirely made up of ciliated cells, and the cilia beat in a coordinated fashion to constantly clear the mucus layer and disseminate immune products [6]. The cilia are also inducible; upstream mechanical or biological changes can speed up ciliary beating and resulting mucociliary clearance (MCC) [7]. Alterations in viscosity or volume of mucus can result in large compensatory changes of MCC [8]. In addition to physical clearance, release of β-defensins (specifically β-defensin 1 and β-defensin 2) in response to microbial products results in direct bacterial killing, as does release of lactorferrin, lysozyme, reactive oxygen species (ROS), or nitric oxide (NO) [9]. All of these immune responses are subject to failure, and the inability to mount a robust innate immune response in the presence of a pathogenic challenge can result in disease.

CRS is a common sinonasal disease with tremendous morbidity comparable to chronic diseases of other organ systems, such as chronic obstructive pulmonary disease (COPD) or congestive heart failure [10]. It affects 16% of the United States population, and accounts for over 10 million physician visits per year [11]. Conventional treatment is on an outpatient basis with antibiotics [12], but increasing bacterial resistance and an overall effort to reduce antibiotic overuse predicate the need for new therapies that leverage endogenous host defense, such as airway receptors. Toll-like receptors (TLR's) respond to bacterial structural or secreted components called pathogen associated molecular patterns (PAMPs) and upregulate expression of genes that bolster the immune system in

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a sustained response to the bacterial insult over several hours [13]. However, increases in MCC and release of antimicrobial compounds are observed almost immediately in response to the presence of bacterial products [14], suggesting that there is an additional independent pathway that is responsible for a more rapid response. T2Rs, responding to bitter bacterial products, appear to mediate this faster pathway.

T2R's are G-protein coupled receptors (GPCRs) that were originally identified in taste bud type II cells [15, 16], and later found to be expressed in several tissues beyond the tongue [17-22]. T2R's were initially localized in the airway in 2009, when Shah *et al*. observed Ca^{2+} responses in bronchial cells that were exposed to bitter compounds [23]. The functions of bitter taste receptors extend far beyond simple taste perception and are still being elucidated, but many of the compounds consciously perceived as bitter on the tongue can be detected in other tissues as well. Compounds detected include denatonium benzoate, sesquiterpene lactones, thujone, absinthin, phenyl-thiocarbamide (PTC) and many others [2, 5, 14, 23-25]. In the tongue, bitter taste receptors prevent ingestion of poisonous or spoiled foods [21, 26], and they appear to play a similar aversive role in the airway.

Activation of a T2R requires complex coordination of cellular second messengers. First, a bitter ligand will bind the GPCR with enough affinity to trigger phospholipase C β2 (PLCβ2). PLCβ2 then causes an increase in inositol trisphosphate (IP_3), which activates an IP_3 receptor on the endoplasmic reticulum (ER), resulting in a stimulated release of calcium (Ca^{2+}) [27]. Separately, the GPCR activation bolsters the ability of phosphodiesterases to reduce cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) activity, augmenting Ca^{2+} release from the ER [26, 28, 29]. All of the released Ca^{2+} activates the transient receptor potential cation channel subfamily M member 5 (TRPM5) channel, which fully depolarizes the cell and triggers ATP release through a separate ion channel, calcium homeostasis modulator 1 (CALHM1) [21, 29-32]. Some bitter taste receptors in the airway have nervous innervation [1, 3, 33] but most act autonomously, transducing local signals and propagating more isolated responses.

T2R38

T2R38, a bitter taste receptor found in both the tongue and airway, has been a focus of innate immunity research over the past several years. T2R38 is found in motile cilia and responds to the bitter compounds PTC and propylthiouracil (PROP) [34], and it also responds to two distinct acyl-homoserine lactone (AHL) products produced by the gram-negative bacterium *Pseudomonas aeruginosa*, N-butyrl-L-homoserine lactone and N-3-oxo-dodecanoyl-L-homoserine lactone [2]. AHL's are secreted by many gram-negative bacteria and serve as quorum-sensing molecules [2, 35-37]. The compounds serve a signaling purpose in the host, allowing the bacteria to communicate information regarding density of colonization [1, 38]. Once bacterial density reaches a substantial level, the bacteria can secrete biofilm matrix and create a stable, highly immune-resistant mass [39]. Bitter taste receptors in the airway allow mammalian hosts to "spy" on these bacterial communications [37], eliciting immune responses before bacteria can reach these infectious levels.

Activation of T2R38 by AHLs, PTC, or PROP results in nitric oxide (NO) production by an inducible nitric oxide synthase [5] downstream of the Ca^{2+} response (Figure **1**) [2]. NO is directly bactericidal, and quickly diffuses out of the ciliated cell to the apical mucus, where it enters the bacterium and and prduces S-nitrosothiols and peroxynitrites that destroys bacterial

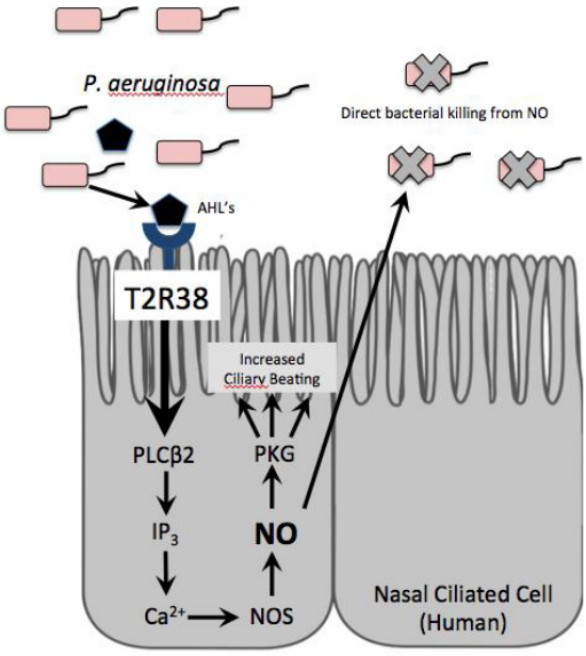

Figure 1: Activation pathway of T2R38. First, AHL's produced by *P. aeruginosa* bind to the functional receptor, which causes an increase in intracellular Ca^{2+} via PLCβ2 and IP_3. This increase in calcium stimulates nitric oxide synthase (NOS) to make NO, which then increases ciliary beating through PKG and diffuses directly into bacteria, where it has potent bactericidal effects.

DNA, membranes, and enzymes [40-42]. In addition to this potent bacterial killing, NO also activates protein kinase G (PKG) and guanylyl cyclase to directly increase CBF and MCC [43]. A series of experiments performed by Lee *et al*. demonstrated that blockade of TRPM5 or PLCβ2, critical components of the taste receptor pathway, completely inhibited T2R38 signaling and resultant NO responses [4, 21, 44]. Furthermore, strains of *P. aeruginosa* that lack the ability to synthesize AHL's do not activate NO secretion via T2R38 in airway epithelial cells [2].

TAS2R38 POLYMORPHISMS IN CHRONIC RHINOSINUSITIS

The diversity of bitter taste receptors is remarkable; humans have at least 25 T2R subtypes, many of which have genetic polymorphisms that can potentially change receptor function [45, 46]. This variety is illustrated by individuals' tastes in food; certain people find bitter foods such as coffee to be abhorrent, while others have non-aversive responses [47, 48]. This variation in function is borne out in the airway as well, with differing sensitivities to bitter products based on genotype.

TAS2R38 is the genetic locus for T2R38, at which there are three commonly-segregating polymorphisms, resulting in a functional receptor (PAV) and a non-functional receptor (AVI). The PAV allele is translated into a receptor protein with a proline, alanine, and valine (PAV) sequence, while the AVI allele results in a receptor protein with an alanine, valine, and isoleucine (AVI). The AVI-containing protein is non-functional and renders the receptor non-responsive to PTC, PROP, or AHLs [48]. Thus, homozygotes at the AVI locus (AVI/AVI patients) cannot taste PTC or PROP on the tongue [49], and AVI/AVI nasal epithelial cells demonstrate significantly reduced NO production in response to AHL's. AVI/AVI patients comprise approximately 30% of the population [48], making the PAV/AVI population proportions roughly equal with Mendelian patterns. Heterozygotes (PAV/AVI patients) maintain at least a partially intact NO response, while cell cultures from PAV/PAV homozygotes exhibit robust stimulation when incubated with AHLs. The lack of NO response from AVI/AVI patients leads to commensurate reductions in bacterial killing and MCC *in vitro*, when compared with cultures expressing a functional PAV receptor [2]. These differences were borne out in *in vivo* studies, as PAV/PAV patients have far less culture positivity for *P. aeruginosa* or other gram-negative bacteria, without significant differences in other T2R genotypes, including T2R19, 30, and 46 [2].

It has long been known that CRS tends to run in families, suggesting a genetic component to the disease [50-52]. Additionally, sinonasal explants from patients with CRS do not show increases in CBF in response to a number of normally ciliostimulatory compounds [53, 54], and NO levels in the airway are significantly different in patients with CRS or other diseases of the airway [55]. This lack of early response, with the resultant presence of chronic infection and unchecked biofilm formation can lead to more serious manifestations of CRS that necessitate surgical treatment [56-58], and the inflammatory conditions found in fulminant CRS can cause augmentation of TNFα and other inflammatory cytokines that further prevent the dynamic response of ciliated cells to mechanical or bacterial stimulation [59, 60]. This vicious cycle can result in full blown collapse of effective clearance mechanisms, resulting in chronic mucus stasis that further harbors pathologic proliferation of bacteria [61], while also allowing ciliotoxic and cytotoxic bacterial secretions to reach dangerous levels [62, 63]. The full pathophysiology behind the differences observed in immune responses in patients with airway diseases has not yet been fully elucidated, but the TAS2R38 genotype appears play a key role.

TAS2R38 polymorphisms have important implications in our understanding of gram-negative infection and CRS, and their clinical impacts have been evaluated in several recent studies. First, a retrospective study of patients who underwent functional endoscopic sinus surgery (FESS) for CRS treatment revealed that 46% of patients having surgery had the AVI/AVI genotype, compared to only 3.6% of patients who were PAV/PAV [64]. This deviated very significantly from the expected genotype distribution, confirming that individuals who can sense AHL's produced by gram-negative bacteria are much less likely to require surgical treatment for CRS symptoms than "non-tasting" patients [49, 64]. This study, by Adappa *et al*., further demonstrated that the PAV/PAV patients are less prone to gram-negative infection, including infection with *P. aeruginosa*. A prospective study in 2014, also by Adappa *et al*., confirmed the significant TAS2R38 genotype skew in CRS patients undergoing FESS for their disease [49].

Testing for TAS2R38 Polymorphisms

T2R38 responds to PTC and PROP in the tongue and sinonasal epithelial cells, and the ability to perceive the taste of PTC on the tongue correlates with expression of functional receptor in the nasal mucosa. Based on initial data, PTC taste testing may be even more specific for the PAV receptor than TAS2R38 genotyping, as it can serve as a proxy for quantity of PAV receptor in the epithelium [65]. Instead of expensive genetic testing of patients, bitter taste testing with PTC or PROP is a cheaper alternative to determine bitter taste phenotype of CRS patients. This information can allow surgeons to potentially stratify surgical candidates most likely to benefit from traditional FESS treatment, while also identifying other patients that need aggressive or alternative management [2].

T2R38 and Biofilm Formation

More recently, the impact that TAS2R38 genotype has on biofilm formation has been studied. Because AHL's function as signaling molecules that aid in density and biofilm communication, patients that lack the ability to detect AHL's before they reach high concentrations would be expected to have a higher propensity towards biofilm development in the airway. A biofilm can serve as a nidus for repeated acute infections [66], and in CRS biofilms are associated with more severe inflammatory disease and poor postoperative outcomes [67-69]. PTC taste sensitivity correlates linearly with *in vitro* biofilm formation; patients with higher PTC taste intensity have epithelial cells that are less likely to harbor *P. aeruginosa* biofilms in culture [65]. The differences observed are driven exclusively by nonpolypoid CRS patients. Cultures from polyp patients showed no correlation between biofilm formation and patient PTC sensitivity, lending evidence to the suggestion that CRS with and without polyps are two fundamentally distinct diseases [70]. This study supports the use of PTC as a proxy for the functionality of the T2R38 receptor exclusively in nonpolypoid patients, and its potential use in clinically-informed decisions.

CONCLUSIONS

The contribution of the T2R38 receptor to medically-recalcitrant CRS is evident, and further investigations of the impact of this receptor on respiratory disease are underway. Patients with a non-functional receptor haplotype (AVI/AVI) have more gram-negative infection, and more often require surgery for CRS. Additionally, sinonasal cultures from AVI/AVI patients are more likely to form biofilms *in vitro*, likely due to the inability to detect AHL's before they reach biofilm-forming concentrations. Receptor polymorphisms have more prominent influence in non-polyp disease, opening up the possibility that there is a yet unidentified class of T2R's that contributes to polyp-based disease.

The implications of T2R38's antimicrobial function in the airway are broad. First, patient genotype can be used to inform clinical decision making, identifying at risk patients as well as therapies that specifically target gram-negative infection. Beyond this, bitter agonists of T2R38 or other taste receptors could serve as therapeutic agents in the airway, leveraging endogenous host defenses. Bitter taste agonists can be used to increase MCC and NO production to amplify innate immunity without antibiotics, potentially preventing proliferation of drug-resistant organisms and recalcitrant infections. Understanding the role that bitter taste receptors play in health and disease will further these aims, and allow for better recruitment of innate immune mechanisms.

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