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REVIEW-SYMPOSIUM

# Chemosensory cells in the respiratory tract as crucial regulators of innate immune responses

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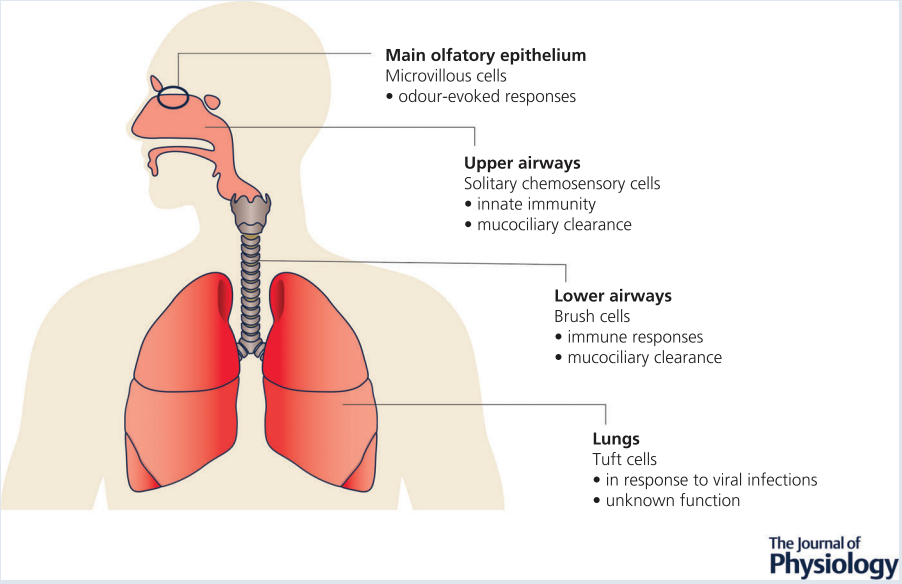


**Abstract** During recent years chemosensory cells in extraoral tissues have been established as mediators for the detection and regulation of innate immune processes in response to pathogens. Under physiological conditions, chemosensory cells are present throughout the respiratory epithelium of the upper and lower airways as well as in the main olfactory epithelium. Additionally, they emerge in the alveolar region of the lung upon viral infections. Chemosensory cells in the upper and the lower airways detect signalling molecules from gram-positive and gram-negative bacteria as well as aeroallergens and fungi. Upon stimulation they release multiple molecules, such as the transmitter acetylcholine, the cysteinyl leukotriene E4 and the

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cytokine interleukin-25, which act as autocrine and paracrine signals and thereby orchestrate the innate immune responses in the respiratory system. Activation of chemosensory cells stimulates various immune cells, e.g. type 2 innate lymphoid cells, modulates mucociliary clearance and induces a protective neurogenic inflammation. This review compiles and discusses recent findings regarding chemosensory cell function in the respiratory tract.

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**Abstract figure legend** Chemosensory cells that are characterised by the expression of a functional bitter taste signalling cascade are widely expressed in tissues outside of taste buds in the tongue. In the respiratory system they are known under diverse names: microvillous cells in the main olfactory epithelium, solitary chemosensory cells in the upper airways, brush cells in the lower airways and tuft cells in the lung. In recent literature, some authors refer to these chemosensory cells generally as tuft cells, regardless of the organ they are expressed in. While little is known about the function of microvillous cells and lung tuft cells, a clear role has been established for solitary chemosensory cells and brush cells in eliciting important innate immune functions after stimulation by metabolites from pathogens. This review discusses recent findings on the function of these chemosensory cells in the respiratory tract.

## Introduction

Recent RNAseq studies have uncovered an enormous heterogeneity among cells of the respiratory epithelium. Besides the well-known cell types, such as ciliated cells, basal cells (progenitor cells), secretory cells and neuroendocrine cells, additional novel cell types, such as ionocytes, have been identified, and others, such as chemosensory cells, have been characterized in more detail. The main characteristics and functions of each of the different cell types have been reviewed in more detail elsewhere (Davis & Wypych, 2021). Ciliated cells represent the biggest cell population of all of them. They can be found throughout the airways and are equipped with motile kinocilia, which play a crucial role in the removal of inhaled xenobiotics out of the airways. In contrast to the more abundant cell types, chemosensory cells as well as neuroendocrine cells and ionocytes represent so-called rare epithelial cell types. In the mouse trachea chemosensory cells comprise around 1% of the total epithelial cell population (Krasteva et al., 2011; Saunders et al., 2013). From their discovery half a century ago until the early 2000s, the cells could be distinguished solely on an ultrastructural level by the presence of an apical tuft of microvilli (Rhodin & Dalhamn, 1956), and therefore were often referred to as 'tuft' or 'tufted' cells. About two decades ago, nasal cells with an apical microvillous tuft were assigned chemosensory functions (Finger et al., 2003). Later on, it became apparent that this is also true for 'tuft' cells at other locations. Despite their distinct morphology, today chemosensory cells can be divided into different subtypes according to their expression profile as discussed below.

During the last decade, it has become evident that chemosensory epithelial cells are present in various other organs, such as the urethra, intestinal mucosa, gallbladder, gingiva, conjunctiva and thymus (Deckmann et al., 2014; Gerbe et al., 2016; Howitt et al., 2016; Keshavarz et al., 2022; Krasteva et al., 2011; O'Leary et al., 2022; Panneck et al., 2014; Saunders et al., 2014; Von Moltke et al., 2016; Wiederhold et al., 2015; Zheng et al., 2019). Unrelated to their above-mentioned locations, strikingly they all share the common feature of detecting such changes in their microenvironment as the presence of pathogens or allergens. Depending on their location, chemosensory cells have been named differently. In the lower airways and in the urethra they are called 'brush cells', in the upper airways 'solitary chemosensory cells', in the main olfactory epithelium 'microvillous cells', and in the intestine, they are referred to as 'tuft cells'. The name 'tuft cell', however, emerges as the preferring name for chemosensory cells at other locations. Chemosensory cells express certain consensus genes, display a similar morphology and play important roles in initiating innate immune processes. Yet, some of these functions depend on the location of these cells. For clarity, it is advisable to specify the location of the chemosensory cells to avoid any confusion. A brief summary is provided in Table 1.

Tracheal brush cells are characterized by the expression of a functional bitter taste signalling cascade comprising bitter taste receptors (Tas2R) coupled to the G protein $\alpha$-gustducin, phospholipase $C_{\beta 2}$ ($PLC_{\beta 2}$) and the transient receptor potential melastatin 5 (Trpm5) ion channel (Finger et al., 2003; Krasteva et al., 2011; Tizzano et al., 2010). Activation of brush cells leads to a

**Table 1. Characteristics of chemosensory cell types of the airways**

| Characteristic | Brush cells | Solitary chemosensory cells | Microvillous cells | Lung tuft cells |
|---|---|---|---|---|
| Location | Lower airways | Upper airways | Main olfactory epithelium | Lungs |
| Examples of signature genes (mouse gene names are listed) | *Trpm5*, *Chat*, *Plcb2*, *Pou2f3*, *Avil*, *Sox9*, *Dclk1*, *Gnat3*, *Alox5ap*, *Alox5* | *Trpm5*, *Chat*, *Plcb2*, *Pou2f3*, *Avil*, *Sox9*, *Gnat3*, *Alox5ap*, *Alox5* | *Trpm5*, *Chat*, *Plcb2*, *Avil*, *Pou2f3* | *Dckl1*, *Trpm5*, *Gnat3*, *Pou2f3*, *Alox5*, *Gnat3*, *Sox9* |
| Examples of expressed receptors | Tas2R, Tas1R, mAChR M1, M2, M3, IL17RB | Tas2R, Tas1R, P2X4, P2Y2, $\alpha$7 nAChR, IL17RB | P2X, Tas1R | Tas2R, Tas1R |
| Sensory innervation | Vagal | Trigeminal | Scarce | Unknown |
| Distinction of subpopulations | Yes | Yes | Yes | Yes |
| Synthesised effector molecules | ACh, CysLT, IL-25 | ACh, CysLT, IL-25, PGE2 | Unknown | Unknown |
| Functions | Detection of bacteria and aeroallergens, neurogenic inflammation, recruitment of ILC2 and dendritic cells, modulation of mucociliary clearance | Detection of pathogens, neurogenic inflammation, recruitment of ILC2 and mast cell degranulation, modulation of mucociliary clearance | Involved in odour-generated responses, e.g. food detection | Unknown |

Trpm5-dependent release of acetylcholine (ACh) from these cells (Hollenhorst et al., 2020; Perniss et al., 2020). This signalling cascade was originally discovered in type II cells of the taste bud, as reviewed by Kinnamon (2012), Lee & Cohen (2015) and Roper & Chaudhari (2017). There, the $G_{\beta\gamma}$ signalling leads to an activation of $PLC_{\beta 2}$ and to a release of $Ca^{2+}$ from intracellular stores, which opens the Trpm5 channel. The resulting $Na^{+}$ influx activates voltage-gated $Na^{+}$ channels generating an action potential that leads to an ATP release via calcium homeostasis modulator 1 and 3 (CALHM1/3) heteromeric channels (Ma et al., 2018). Yet, while key components of this bitter taste signalling cascade, such as the Trpm5 channel, have been demonstrated to be functional in brush cells, solitary chemosensory cells and tuft cells, it remains to be shown, whether the signal transduction pathway in these cells involves additional canonical taste signalling components. While type II taste cells as well as tracheal brush cells and most probably nasal solitary chemosensory cells are able to release ACh (Dando & Roper, 2012; Hollenhorst et al., 2020; Perniss et al., 2020; Saunders et al., 2014), an ATP release from chemosensory cells in the respiratory tract has not been shown yet.

In the following sections, this review will focus on recent findings and addresses questions regarding the function of chemosensory cells of the respiratory tract.

## Brush cells in the lower airways

In recent years, several sequencing studies of tracheal brush cells from mouse and human airways have revealed a distinct cell population consisting of different subpopulations (Bankova et al., 2018; Deprez et al., 2020; Goldfarbmuren et al., 2020; Montoro et al., 2018; Nadjsombati et al., 2018; Plasschaert et al., 2018). A member of the bitter taste receptors, *Tas2R108*, was identified as a gene constitutively transcribed in all subpopulations (Bankova et al., 2018; Montoro et al., 2018; Nadjsombati et al., 2018). The taste transduction protein Trpm5 was identified as a marker protein for all these cells. Montoro et al. (2018) distinguished two different mature populations of brush cells in the mouse trachea that they termed 'tuft-1' and 'tuft-2' cells, since in recent nomenclature the term 'tuft' cell can be used not only to denote chemosensory cells in the intestine, but also as a more general term for chemosensory cells in extraoral tissues. A third cluster of chemosensory cells represented an immature cell population. Tuft-1 cells further expressed genes for taste transduction, such as *Gnat3* and *Plcb2*, while tuft-2 cells expressed genes that are associated with leukotriene synthesis, such as *Alox5ap* (Montoro et al., 2018). The transcription factor Pou2f3 has been identified as a marker for tuft-1 cells, and Sox9 for tuft-2 cells. Pou2f3 is required for

chemosensory cell development and its deletion led to absence of TRPM5$^+$ brush cells in the tracheal epithelium (Perniss et al., 2020). Additionally, Montoro et al. (2018) confirmed observations made in other studies that brush cells were cholinergic (Bankova et al., 2018; Hollenhorst et al., 2020; Nadjsombati et al., 2018) and identified *Chat* gene expression as characteristic for the subset of the tuft-1 cell population. Findings by Bankova et al. (2018) contradict the classification into tuft-1 and tuft-2 cells introduced by Montoro et al. (2018). In their study, Bankova et al. (2018) analysed *Chat* expressing cells and detected the transcription of genes associated with leukotriene synthesis, such as *Ltc4s*, *Alox5* and *Alox5ap*, which were assigned to the tuft-2 cell population by Montoro et al. (2018). Independent of the classification of the choline acetyltransferase (ChAT)$^+$ cell population, high expression of interleukin (IL)-25, an inflammatory cytokine important for eliciting type 2 immune responses, was found in both groups of cells, pointing towards their significance as a trigger of innate immune responses (Bankova et al., 2018; Montoro et al., 2018). In conclusion, it appears obvious that different subpopulations are present, but a more detailed characterisation in the future will be needed in order to identify signature genes transcribed in different brush cell populations. The gene signatures might vary depending on the activation of the cells and be representative of the functional status of the cells.

In humans, chemosensory cells are present in the upper as well as in the lower airways, with a higher proportion in the distal bronchi than in the nose (Deprez et al., 2020). While Deprez and colleagues noted clear differences in the gene expression profile of some more abundant cell types, such as ciliated and secretory cells, between the nose and the tracheobronchial system, they did not address possible differences in the transcriptional profile of chemosensory cells with different locations in the respiratory tract. In the human trachea, brush cells were found to be a distinct cell population (Deprez et al., 2020), whereas in human bronchial primary air–liquid interface epithelial cultures, brush cell genes clustered with those of neuroendocrine cells (Plasschaert et al., 2018). There might be several explanations for these differences, e.g. differences between the chemosensory cells isolated from different airway regions (tracheal *vs*. bronchial cells), differences due to culturing of the epithelial cells *vs*. fresh tissue, or differences in the sequencing protocols. Future studies will be needed to shed light on this important issue. Another study analysing fresh human tracheal tissue samples delineated a cell population that expressed *Pou2f3*, *Ascl2*, *Lrmp*, *Myb*, *Il23A* and *Arl9* (Goldfarbmuren et al., 2020). Since the tuft cell markers *Gnat3* and *Trpm5* were absent and the cells clustered with ionocytes, the authors classified these cells as 'tuft-like'. In contrast to previous work by Plasschaert et al. (2018), who used human bronchial cell cultures, the neuroendocrine cells represented a distinct cell population in the study of Goldfarbmuren et al. (2020). Thus, the signatures of the human airway chemosensory cells change along the respiratory tree and it appears plausible that this will reflect also differences in their function. The study of Plasschaert and colleagues is limited by its cultural approach, in which rare cell populations might not be completely differentiated and exhibit mixed features. Inactivation of *Pou2f3* by CRISPR/CAS in human tracheal epithelial cultures abolished the development of tuft-like cells and reduced the numbers of ionocytes and neuroendocrine cells, leading Goldfarbmuren et al. (2020) to conclude that tuft-like cells potentially serve as progenitors for neuroendocrine cells as well as for ionocytes. However, this conclusion does not entirely rule out other possible sources of origin.

In mice deficient for *Pou2f3*, tracheas lack only brush cells (Perniss et al., 2020). This might be due to the fact that in mice brush cells are found only in extrapulmonary airways and that the bronchial tuft or tuft-like cell population is not present. In mice, tracheal brush cells surface during embryonic development at E18 and expand postnatally in a sex-independent manner. This is in contrast to urethral chemosensory cells (Perniss et al., 2021). The transcription factor Pou2f3 is essential for chemosensory cell development in the airways as well as in the intestine (Gerbe et al., 2016; Perniss et al., 2020). In adult mice, tracheal brush cells build a stable cell population under non-stimulatory conditions. Under basal conditions, 5-bromo-2′-deoxyuridine and Ki67 labelling were only present in other epithelial cell types but not in brush cells, although brush cells were clearly present in the epithelium (Saunders et al., 2013). Till today, the progenitors of airway brush cells have yet not been demonstrated unequivocally. The study from Montoro et al. (2018) suggested that the rare airway epithelial cell types in the mouse trachea including tuft cells, neuroendocrine cells and ionocytes originate from cytokeratine-5$^+$ basal cells, since a lineage tracing of cytokeratin-5-expressing cells labelled tuft cells, whereas the Scgb1a1 (a club cell marker) lineage-labelled population showed only a few tuft cells. Although the number of Scgb1a1 lineage-labelled cells was sparse, a small population of tuft cells seems to be derived from club cells. Interestingly, the airway chemosensory cell population expands under pathophysiological conditions. It arises from the same progenitor cell populations and the development involves the same transcription factor, Pou2f3. Viral infections led to the appearance in the lung of ectopical tuft cells (described below), which were not present when Pou2f3 was depleted. These ectopical tuft cells derived from basal cells (cytokeratin-5$^+$ and Trp63$^+$) and to a minor extent from club cells (Huang et al., 2022). Tracheal brush cells expanded also in response to inhaled allergens. The expansion was mediated by cysteinyl leukotriene $LTE_4$ and dependent on the cytokine

IL-25, which are both secreted by brush cells (Bankova et al., 2018). Thus, it seems that the expansion of airway chemosensory cells is autoregulated and triggered by the presence of certain environmental stimuli. It this context, it would be of particular interest to reveal if the expansion of the chemosensory cell population is beneficial or harmful.

## Mediators released from tracheal brush cells

**Acetylcholine.** The key functions of tracheal brush cell activation that are discussed in the following paragraphs are summarised in Fig. 1. The expression of components of the bitter taste signalling cascade such as Trpm5 as well as the synthesising enzyme for ACh, ChAT, were suggestive of functional implications for the activation of tracheal brush cells in mice (Kaske et al., 2007; Krasteva et al., 2011). Indeed, stimulation of brush cells with bacterial quorum-sensing molecules or the bitter substance denatonium decreased the respiratory rate and induced a cough-like reflex in mice (Hollenhorst et al., 2020; Krasteva et al., 2012). This effect was abrogated when the epithelial layer was mechanically abraded and nicotinic ACh receptors were inhibited with mecamylamine, which pointed towards a release of ACh from a non-neuronal epithelial source. Direct proof of a release of ACh from mouse brush cells was provided 2 years ago. The release involved the activation of key components of the bitter taste signalling cascade, notably the Trpm5 ion channel (Hollenhorst et al., 2020; Perniss et al., 2020). Moreover, ACh released from brush cells had an autocrine effect on brush cells themselves, by augmenting bitter taste stimulus-induced $[Ca^{2+}]_i$ increase in brush cells via the muscarinic ACh receptors M1 and M3 and by reducing $[Ca^{2+}]_i$ in brush cells via M2 receptors (Hollenhorst et al., 2020).

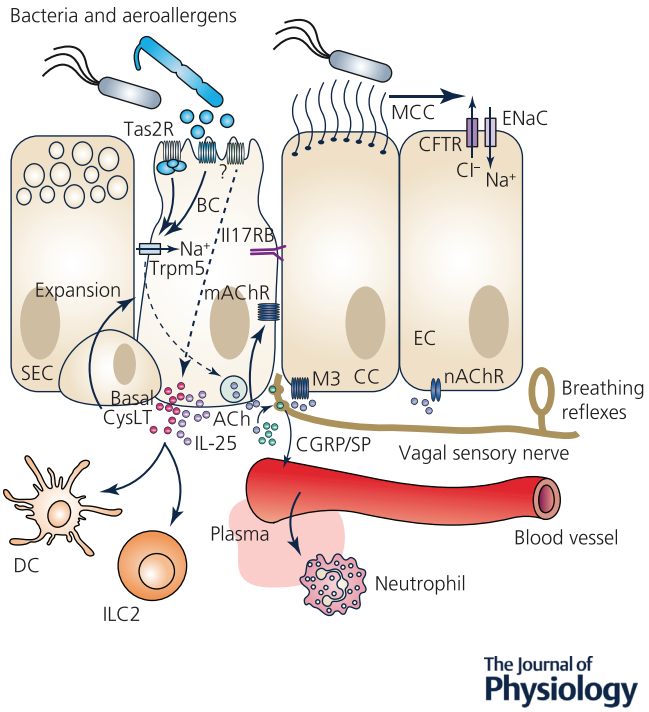


**Figure 1. Brush cells in the lower airways**
Brush cells (BC) can be activated by bacterial molecules or aeroallergens. This elicits a Trpm5-dependent release of acetylcholine by an unknown mechanism as well as a release of cysteinyl leukotrienes and the cytokine IL-25 of which the mechanism likewise is not elucidated. These signalling molecules then evoke different innate immune responses, such as a recruitment of dendritic cells (DC) and innate lymphoid type 2 cells (ILC2) via the cysteinyl leukotriene (CysLT) $LTC_4$ as well as a neurogenic inflammation mediated by calcitonin gene-related peptide (CGRP) and substance P (SP) release from sensory nerve endings resulting in blood vessel dilatation, plasma extravasation and neutrophil recruitment via acetylcholine. Acetylcholine further stimulates mucociliary clearance (MCC), signals back to brush cells in an autocrine loop and induces protective breathing reflexes. Additionally, cysteinyl leukotriene release leads to brush cell expansion. basal, basal cell; CC, ciliated cell; EC, epithelial cell; SEC, secretory cell.

**Leukotriene C4 and interleukin 25.** Recent studies in mice revealed that brush cells are the major source of IL-25 in the airways and also produce the cysteinyl leukotriene C4 ($LTC_4$) (Bankova et al., 2018; Ualiyeva et al., 2021). Brush cells synthesised $LTC_4$ upon stimulation with allergens (Ualiyeva et al., 2021), and evoked an innate type 2 immune response, which involved dendritic cells and innate lymphoid type 2 cells (ILC2). Interestingly, the effect of IL-25 and cysteinyl leukotrienes was synergistic and led to a higher recruitment of dendritic cells and ILC2 cells when both substances were administered simultaneously (Ualiyeva et al., 2021). Besides their response to brush cell-derived cysteinyl leukotrienes and IL-25, dendritic cells themselves are a source of cysteinyl leukotrienes (Barrett et al., 2009), and thus could further amplify the effects of brush cell activation, since brush cells express the cysteinyl leukotriene receptor 3 (Bankova et al., 2018). Moreover, it is possible, that not only $LTC_4$ is responsible for ILC2 recruitment and activation, since ILC2 express also muscarinic ACh receptors subtypes M4 and M5 as well as the nicotinic receptor subunits $\alpha 2$, $\alpha 5$, $\alpha 9$, $\alpha 10$, $\beta 1$ and $\beta 2$ (Chu et al., 2021). The ACh receptor expression is regulated by the brush cell-derived cytokine IL-25. Stimulation of muscarinic and nicotinic ACh receptors in ILC2 cells induced IL-13 expression in ILC2 cells (Chu et al., 2021) and the recruitment and activation of eosinophils (Chu et al., 2021). In addition, not only can ILC2s be activated by ACh but they might also synthesise ACh, since these cells were found to be eGFP+ in ChAT-eGFP mice infected with the nematode *Nippostrongylus brasiliensis* (Roberts et al., 2021). The direct proof of ACh release from ILC2 cells is yet to

be provided. Nevertheless, this finding is very intriguing since under physiological conditions, only brush cells were found to be ChAT-eGFP$^+$ in the mouse airways. It remains to be elucidated if ILC2 cells express ChAT also under pathophysiological conditions other than nematode infection. It is tempting to speculate that ILC2-derived ACh may act on brush cells in either a stimulatory (M1 and M3 ACh receptor-dependent) or an inhibitory (M2 ACh receptor-dependent) way dependent on the receptor expression and, thus, increase or terminate the brush cell-induced immune responses.

Besides the cholinergic autocrine signalling loop, an autocrine signalling loop for brush cell-released IL-25 has been proposed, since brush cells express the IL-25 receptor IL17RB (Bankova et al., 2018). Although this still awaits experimental confirmation for airway brush cells, an IL-25-dependent feedback mechanism in the epithelium has been established for intestinal tuft cells. IL-25 released from intestinal tuft cells induced IL-13 release from ILC2 cells, which in turn stimulated epithelial progenitor cells and, consequently, increased the number of tuft cells (Von Moltke et al., 2016).

While it is well recognised that brush cells release ACh, IL-25, and cysteinyl leukotrienes, the release mechanisms remain to be elucidated. It might also be of interest to further pursue the question of whether the different mediators are released simultaneously in the airways, as has recently been suggested for cysteinyl leuktorienes and ACh in tuft cells of the gallbladder (Keshavarz et al., 2022), or if the release involves a distinct pathway for each of them. A simultaneous release or a co-release would implicate a concerted action of the mediators, whereas a release upon specific stimuli would indicate a fine-tuned modulation of distinct responses. Pointing towards a synergistical action of all three mediators are findings from Hollenhorst, Nandigama et al. (2022), who reported that the ILC2 cytokine IL-5 was upregulated after a bacterial infection.

## Brush cell functions

**Regulation of mucociliary clearance.** Interestingly, several bacterial substances, such as formyl peptides or *Pseudomonas aeruginosa* quorum-sensing molecules of the *Pseudomonas* quinolone signal or the acyl-homoserine lactone quorum-sensing system, can also be recognised by mouse brush cells and trigger protective responses such as an induction of protective breathing reflexes or an increase in mucociliary clearance (Hollenhorst et al., 2020; Krasteva et al., 2012; Perniss et al., 2020). In general, mucociliary clearance consists of three main components: ciliary beating, production of mucus and production of airway surface liquid. All of them are functionally important to maintain airway homeostasis and prevent respiratory diseases. In mouse tracheas, quorum-sensing molecules and formyl peptides stimulated the mucociliary clearance by stimulation of ciliary beating and the flow of particles on the tracheal surface dependent on Trpm5 activation in brush cells. However, while the *P. aeruginosa* quorum-sensing molecules C12-oxohomoserine lactones are known to be agonists of the bitter taste receptors Tas2r105 and Tas2r108, which are abundantly expressed in brush cells, the receptor responsible for the detection of the formyl peptides containing the core motif MKKFR in brush cells remains to be identified (Hollenhorst, Nandigama et al., 2022; Perniss et al., 2020). Interestingly, the stimulation of mucociliary clearance by formyl peptides was not mediated by formyl peptide receptors or by bitter taste receptors (Perniss et al., 2020). This indicates that brush cells possess a wide spectrum of detection, most probably also through to-date-unidentified receptors, allowing them to respond to a broad variety of environmental substances. Similar to tracheal brush cells, the chemosensory cells of the upper respiratory tract, e.g. solitary chemosensory cells of the mouse nasal epithelium as well as human sinonasal epithelial cultures, responded to bacterial quorum-sensing molecules with an increased ciliary beat frequency and mucociliary transport (Lee et al., 2012; Lee, Chen et al., 2014).

An optimal volume (critical thickness) of the airway surface liquid is needed for the maintenance of a functional ciliary beat, which results in the transport of potentially harmful agents out of the airways. Recently, Hollenhorst, Kumar et al. (2022) have shown that the activation of mouse tracheal brush cells by denatonium not only has an influence on the cilia-mediated part of the mucociliary clearance, but also is involved in the regulation of the airway lining fluid secretion. The denatonium-induced changes in transepithelial ion transport involved two signalling pathways. First, there was an activation of the classic bitter taste signalling cascade including the $G_{\beta\gamma}$-subunit of the G protein-coupled taste receptors, phospholipase $C_{\beta 2}$, $IP_3$ receptors, Trpm5 and a release of ACh. This resulted in a paracrine inhibition of the epithelial sodium channel (ENaC) via nicotinic ACh receptors decreasing apical reabsorption of sodium and thereby increasing the amount of airway lining fluid. Second, the $G_\alpha$-dependent pathway was activated leading to decreased intracellular cAMP levels via an inhibition of adenylyl cyclase, resulting in a paracrine inhibition of the apical cystic fibrosis transmembrane conductance regulator (CFTR) as well as of the basolateral $Na^+$–$K^+$–$2Cl^-$ cotransporter (NKCC1) and the KCNQ1 potassium channel. This leads to a decreased secretion of apical chloride and thereby a reduced secretion of airway lining fluid (Hollenhorst, Kumar et al., 2022). Thus, brush cells are most probably involved in the maintenance of the homeostasis of airway lining fluid secretion.

While brush cells can regulate the ciliary beat as well as the transepithelial fluid transport, it needs to be demonstrated whether brush cells are also involved in the regulation of mucus production and secretion. A recent study in the intestine identified an increased secretion of mucus from goblet cells due to the activation of the vomeronasal olfactory receptor Vmn2r26 by the *Shigella* metabolite *N*-undecanoylglycine in tuft cells and the release of prostaglandin D2 (Xiong et al., 2022). Yet, while expression of these Vmn2r26 receptors remains to be shown in the airway epithelium, a recent study describes synthesis of the prostaglandin E2 by nasal chemosensory cells (Kotas et al., 2022). In this context, it appears plausible that brush cells might also be involved in promoting mucus secretion in the airways and thereby regulate all three parts of mucociliary clearance.

**Resolution of inflammation.** To date, it seems clear that brush cells play a crucial role in inducing immune responses. However, an overshooting and persisting immune response is in general harmful (Rossi et al., 2021). It is tempting to speculate that brush cells can regulate the inflammatory response by limiting their own activity, e.g. through an inhibitory autocrine feedback loop via ACh (Hollenhorst et al., 2020). This might lead to a switch from a pro-inflammatory to an anti-inflammatory or to an inflammation resolving state. Supportively, peptides that are involved in a negative regulation of endopeptidase activity, such as serpins, as well as peptides involved in an activation of the immune response were among the most abundant peptides in recently performed proteome analyses of supernatants collected from tracheas after brush cell stimulation (Hollenhorst, Nandigama et al., 2022). Serpins can inhibit the function of neutrophil serine proteases, which are important for preventing damage caused by the activity of segregated proteases after neutrophil activation and thus help to prevent an overshooting immune response. Remarkably, chemosensory (tuft) cells in the mouse colon synthesise the pro-resolving lipid mediator resolvin D1 and express 5-lipoxygenase (Lox5), an enzyme important for resolvin D1 as well as for leukotriene synthesis (Grabauskas et al., 2022). In favour of a synthesis of inflammation resolving lipid mediators such as resolvin D1 in chemosensory cells in the airways is the finding that Lox5 has been detected in brush cells of the lower airways as well as in solitary chemosensory cells of the upper airways in single cell transcriptome analyses (Ualiyeva et al., 2020). The possible synthesis of inflammation-resolving molecules as well as the induction of inflammation-limiting serpins indicates that chemosensory cells might play an important role in terminating the inflammatory response when the pathogens have successfully been eliminated. Moreover, it is likely that airway chemosensory cells not only play a role in orchestrating the immune system in responses to acute stimuli, but also regulate immunity under homeostatic conditions. This assumption is supported by a recent study on biliary tuft cells that found an enrichment in inflammatory genes and increased neutrophil recruitment in the gallbladder of tuft cell-deficient mice, leading the authors to suggest a limitation of inflammation by these cells under homeostatic conditions (O'Leary et al., 2022).

**Neurogenic inflammation and protective response to bacterial infection.** Remarkably, brush cells are not only involved in inducing innate immune responses to stimulation with allergens as mentioned above (see 'Leukotriene C4 and interleukin 25'), but also in bacterial infections. Recently, Hollenhorst et al. were able to show that activation of tracheal brush cells in mice induces protective neurogenic inflammation as an acute response to brush cell stimulation with bitter substances and bacterial metabolites (Hollenhorst, Nandigama et al., 2022). Neurogenic inflammation also occurs after solitary chemosensory cell activation in the upper airways (Saunders et al., 2014) and this issue will be discussed below. The brush cell-induced neurogenic inflammation was characterised by vasodilatation of capillaries and postcapillary venules, neutrophil recruitment, and plasma extravasation. These processes were mediated by cholinergic signalling, since they were abolished upon inhibition of ACh receptors. Furthermore, the brush cell-mediated neurogenic inflammation involved a release of the neuropeptides calcitonin gene-related peptide (CGRP) and substance P. However, CGRP seems to be the main mediator accounting for the responses, as the neurogenic inflammation was completely abolished by the intraperitoneal administration of the $CGRP_{8–37}$ peptide prior to brush cell stimulation. Since a wide variety of immune cells, such as neutrophils, monocytes and macrophages, express CGRP receptors (Kim & Granstein, 2021), the recruitment of these immune cells was prevented by the inhibition of the CGRP receptors after $CGRP_{8–37}$ administration. In support, in a *P. aeruginosa* infection model, Trpm5-deficient mice, in which brush cell-mediated release of CGRP from neurons does not occur, showed less recruitment of neutrophils, monocytes and alveolar macrophages (Hollenhorst, Nandigama et al., 2022). Additionally, the immune cell diapedesis was likely prevented by the inhibition of CGRP receptors on blood vessel endothelial cells (Tuo et al., 2013).

Moreover, this neurogenic inflammation was especially dependent on the Trpm5 channel and cholinergic signalling to transient receptor potential ankyrin 1 channel $(Trpa1)^+$ sensory neurons. Eighty per cent of these neurons were also transient receptor potential vanilloid 1 channel $(Trpv1)^+$. They originate mainly

from the vagal jugular–nodose–ganglionic complex and from the dorsal root ganglia. As reviewed elsewhere in more detail, the sensory innervation of the airways is crucial for the regulation of respiratory physiology and pulmonary defence (Mazzone & Undem, 2016). According to their physiological responsiveness, different neuronal subtypes exist. The Trpa1$^+$ neuronal fibres comprise unmyelinated C-fibres and can detect a wide range of chemical stimuli (Mazzone & Undem, 2016). Most of them contain the neuropeptides CGRP and substance P (Hollenhorst, Nandigama et al., 2022), and a subpopulation of the peptidergic neurons innervating the airways express the nicotinic acetylcholine receptor $\alpha$3 (Krasteva et al., 2011). Indeed, brush cell-mediated neurogenic inflammation involved cholinergic signalling to Trpa1$^+$ sensory neurons since inhibition of acetylcholine receptors with the agonists mecamylamine and atropine and depletion of Trpa1$^+$ sensory neurons abolished the responses (Hollenhorst, Nandigama et al., 2022). Yet, at high concentrations the bitter substance denatonium evoked a moderate response in mice with depleted Trpa1$^+$ sensory innervation. The residual response is most probably due to the direct stimulation of taste receptors in neurons (Hollenhorst, Nandigama et al., 2022). However, the exact signalling pathway responsible for the release of neuropeptides from Trpa1$^+$ (and Trpa1$^+$/Trpv1$^+$) sensory neurons after stimulation of neuronal ACh receptors remains to be demonstrated. Whether Trpa1 and/or Trpv1 activity is necessary to induce a protective neurogenic inflammation needs also to be addressed in future studies.

Interestingly, the evoked Trpm5-dependent neurogenic inflammation was not exclusively induced by bitter substances such as denatonium but also by supernatants and various quorum-sensing molecules from the gram-negative bacterium *P. aeruginosa* and supernatants from the gram-positive bacterium *Streptococcus pneumoninae*. This further underlines the importance of brush cells as general 'sensors' of bacterial invasion essential for the initiation of rapid innate immune responses. Supportive of the assumption that brush cells are able to recognise a wide spectrum of bacteria is the finding that the core motif MKKFR of formyl peptides that was shown to be important for the increase in particle transport (Perniss et al., 2020) was found in peptides from diverse pathogens of the respiratory tract, such as *S. pneumoniae*, *P. aeruginosa*, *Acinetobacter baumannii*, *Bordetella bronchiseptica*, *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Chlamydia pneumoniae*. In a *P. aeruginosa* infection mouse model with the patient isolate NH57388A, a functional Trpm5 channel was, indeed, necessary to improve survival outcome, weight maintenance, as well as for cytokine production and immune cell recruitment, especially for the recruitment of neutrophils, natural killer cells, monocytes and alveolar macrophages (Hollenhorst, Nandigama et al., 2022). In contrast, Baral et al. (2018) found no differences between mice with intact and ablated Trpv1$^+$ innervation in a lethal *P. aeruginosa* (PA01V) infection model. Moreover, in a severe infection model with the gram-positive bacterium *Staphylococcus aureus*, depletion of Trpv1$^+$ sensory neurons even led to a stronger neutrophil recruitment (Baral et al., 2018). The differences in the neuronal responses in *P. aeruginosa* infection in both studies are most probably due to the different *P. aeruginosa* strains that we used. The NH57388A *P. aeruginosa* strain is a mucoid isolate from a patient suffering from mucoviscidosis and causes a relatively mild course of infection in wild-type mice (Norman et al., 2016). Taken together these findings indicate that the brush cell-mediated responses to pathogens are largely depended on the stage and severity of infection. While brush cells appear to be important at the onset of bacterial infections, they might be incapable of eliciting responses at high bacterial loads or at later stages of severe acute infections. Yet, it is also possible that the findings in severe *S. aureus* infections of Baral et al. (2018) are due to a direct activation of the sensory neurons. In line with this, several studies showed a direct action of *S. aureus* on sensory nerves, leading to *S. aureus*-induced pain (Blake et al., 2018; Chiu et al., 2013). A similar observation was made for the bitter taste receptor agonist denatonium. While at a concentration of 1 mM denatonium induced an epithelium-mediated protective neurogenic inflammation, at 20 mM it directly activated sensory neurons and induced a high inflammatory response (Hollenhorst, Nandigama et al., 2022). In summary, these findings point towards brush cells being the first sentinels for the presence of pathogens, detecting already low doses of bacteria, evoking innate immune responses before the development of a severe infection.

Notably, brush cells seem to combat bacterial infections not only by a recruitment of immune cells that are able to kill bacteria, such as neutrophils, but also by increasing components that display antimicrobial activity. Stimulation of tracheal brush cells with the bitter substance denatonium led to a Trpm5-dependent release of components of the complement system, mainly the complement component C3 (Hollenhorst, Nandigama et al., 2022). The diverse functions and roles of C3 in the host immune response are reviewed in detail elsewhere (Ricklin et al., 2016). Very briefly, C3 conversion into the C3a and C3b components is a key step of complement activation, with C3a displaying antimicrobial activity against gram-negative bacteria and C3b being able to opsonise the pathogens (Andersson Nordahl et al., 2004; Ricklin et al., 2016). In support, C3 augmentation was impaired in Trpm5$^{-/-}$ mice after infection with *P. aeruginosa* and bacterial growth was impaired only in cultures treated with supernatants collected from tracheas

of Trpm5$^{+/+}$ mice after brush cell stimulation. Thus, upregulation of C3 after brush cell activation further underlines the essential role of brush cells in mediating immune responses.

**Role in cancer.** Besides being important in eliciting protective immune functions, brush cells have been suggested to play a role in the development of a subtype of small cell lung cancer (SCLC) in humans that often arises from bronchial epithelial cells (Huang et al., 2018). In tumour samples from patients, the transcription factor Pou2f3, which is essential for the development of chemosensory cells (see also 'Brush cells in the lower airways'), was detected. In support, Pou2f3$^{+}$ cells were found up to the level of the secondary bronchi. Recently, it was shown that the transcription factors OCA-T1 (*C11orf53*) and OCA-T2 (*COLCA2*) are essential for Pou2f3-induced expression of tuft cell specific genes, and that this interaction between Pou2f3 and both coactivators was needed for development of SCLC in cell culture models (Wu et al., 2022). Additionally, the OCA-T1 was identified in a subset of SCLC patient samples and correlated with Pou2f3 expression (Szczepanski et al., 2022). Thus, brush cells might be involved in the development of at least a subset of SCLC, since Pou2f3 expression was detected in 12% of samples from SCLC patients (Baine et al., 2022). Interestingly, these samples showed low expression of neuroendocrine markers, further pointing towards a brush cell origin of this type of cancer. However, the exact role of brush cells in the development of this cancer and their potential pharmacological targeting to suppress tumour growth still necessitates further investigation.

## Solitary chemosensory cells in the upper airways

Analogous to tracheal brush cells, solitary chemosensory cells in the nasal and sinonasal epithelia of mice and humans are chemosensory and express a functional bitter signalling cascade, which can be activated by bitter substances (in humans and mice) and bacterial quorum-sensing molecules (thus far only shown in mice). Activation of the bitter signalling cascade then prompts protective innate immune responses (Finger et al., 2003; Lee, Chen et al., 2014; Lee, Kofonow et al., 2014; Saunders et al., 2014; Tizzano et al., 2010). The major effects of solitary chemosensory cell activation on innate immunity are summarised in Fig. 2. Solitary chemosensory cells in mice also express *Chat* and other marker genes for chemosensory cells, such as *Pou2f3*, *Trpm5*, *Plcb2*, *Avil* and *Sox9* (Ualiyeva et al., 2020). In humans, solitary chemosensory cells have been detected in primary sinonasal cultures and in different regions of the sinonasal cavities, the inferior and middle turbinate, the septum as well as the anterior ethmoid sinus (Chen et al., 2019; Lee, Kofonow et al., 2014). In healthy human turbinate tissue, the solitary chemosensory cells identified by their immunofluorescence for the G protein $\alpha$-gustducin are innervated by peptidergic nerve fibres containing CGRP (Deng et al., 2020). However, $\alpha$-gustducin (Gnat3) was also found in ciliated cells of the lower airways in humans (Shah et al., 2009). Thus, $\alpha$-gustducin expression alone is not sufficient to discriminate these cells as solitary chemosensory cells. However, the $\alpha$-gustducin-labelled cells found by Deng et al. (2020) also expressed the chemosensory cell marker ChAT as well as the $\alpha$7 nicotinic ACh receptor. This is suggestive of the presence of an autocrine cholinergic feedback loop mediated by nicotinic acetylcholine receptors similar to that found in mouse tracheal brush cells, in which an autocrine feedback loop via muscarinic ACh receptors was described (Deng et al., 2020; Hollenhorst et al., 2020). In support, treatment of

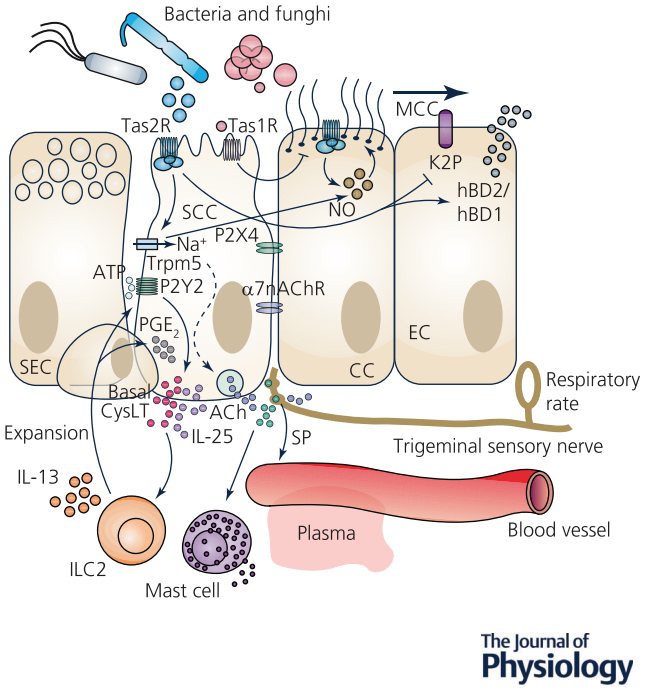


**Figure 2. Solitary chemosensory cells in the upper airways**
Solitary chemosensory cells (SCC) detect different metabolites from gram-positive and gram-negative bacteria as well as from fungi. Activation of the bitter receptors leads to neurogenic inflammation, mediated by acetylcholine and substance P (SP), characterised by plasma extravasation and mast cell degranulation as well as to an increase in $\beta$-defensin production and a modulation of transepithelial ion transport. Mucociliary clearance (MCC) can be stimulated via NO after bitter receptor activation, whereas sweet taste receptor activation leads to an inhibition of mucociliary clearance. Stimulation of solitary chemosensory cells with ATP leads to a release of cysteinyl leukotrienes (CysLT). These cells also release IL-25 and their activation results in innate lymphoid type 2 cell (ILC2) recruitment, which release IL-13 that acts in a feedback loop on expansion of solitary chemosensory cells and prostaglandin E2 production. basal, basal cell; CC, ciliated cell; EC, epithelial cell; SEC, secretory cell.

the human turbinate tissue with a combination of nicotine and IL-13 (see also 'Leukotriene C4 and interleukin 25') increased expression of *Gnat3* and *Trpm5* genes as well as the number of $\alpha$-gustducin and ChAT double-positive cells (Deng et al., 2020). Thus, expansion of solitary chemosensory cells in humans might be regulated by an autocrine feedback loop via ACh or by IL-13 released upon ILC2 stimulation (most probably by ACh). An ILC2 release of IL-13 is observed in intestinal tuft cells in mice (Von Moltke et al., 2016). In line with this, airway chemosensory cells in the nasal as well as in the tracheal epithelium of mice expanded after stimulation with ILC2 cytokine IL-13 (Kotas et al., 2022).

Solitary chemosensory cells in humans play a role in the pathophysiology of chronic rhinosinusitis with nasal polyps. Under these conditions solitary chemosensory cells have been identified as a prominent source of IL-25. In response to the bitter substance denatonium, the number of IL-25$^{+}$ solitary chemosensory cells increased (Civantos et al., 2021). In support, solitary chemosensory cells and ILC2 were enriched in polyps from patients (Kohanski et al., 2018; Patel et al., 2018). In response to two different mould fungi, solitary chemosensory cells in polyps expanded and secreted higher levels of IL-25 (Patel et al., 2019). This was confirmed in a recent study by single cell sequencing of solitary chemosensory cells obtained from nasal polyps of sinus tissue samples from patients with type 2 inflammation, which showed an upregulation of the expression of several human genes specific for solitary chemosensory cell such as *IL17RB*, *TRPM5*, *GNG13*, *ALOX5* and *IL13RA1* (Kotas et al., 2022). The authors also found that the IL-13-induced emerging solitary chemosensory cell population showed an expression of genes detected in solitary chemosensory cells of nasal samples from patients with chronic rhinosinusitis with nasal polyps. Further, the emerging solitary chemosensory cell population produced prostaglandin E2, which led to a CFTR-dependent fluid secretion and increase in mucociliary transport (Kotas et al., 2022).

## Functions of solitary chemosensory cells

**Influence on mucociliary clearance.** The role of bitter taste signalling in innate immunity in the upper airways has been reviewed in more detail by Lee and coworkers (Carey & Lee, 2019; Freund & Lee, 2018). In the following, we will point out a few recent findings. Most of the human studies are performed in air–liquid interface cultures originating from tissue samples of patients with nasal polyps in the ethmoid sinus undergoing sinonasal surgery due to a chronic rhinosinusitis. In human nasal epithelial cultures the bitter substance denatonium impacted the mucociliary clearance by regulating ion transport processes (Kohanski et al., 2021). It activated apical two-pore potassium (K2P) channels in epithelial cells via an $\alpha$-gustducin-mediated decrease in cAMP (Kohanski et al., 2021). Since K2P channels were found to be expressed abundantly in several epithelial cell types in the respiratory mucosa (Zhao et al., 2012), the exact epithelial cell type in which the channels are activated and the channel subtype responsible for the effect remain to be determined. The involvement of solitary chemosensory cells in the observed ion transport changes needs further evidence, since besides pertussis toxin, which in addition to $\alpha$-gustducin also inhibits $G_{\alpha i}$ subunits, no other inhibitors of the bitter signalling cascade were used.

According to older studies in humans as well as in mice, detection of bitter substances cannot only be attributed to solitary chemosensory cells, since ciliated cells have also been shown to express bitter taste receptors and to be activated by bitter compounds in the upper as well as in the lower airways (Lee et al., 2012; Lee, Chen et al., 2014; Shah et al., 2009). In humans, activation of bitter taste receptors plays a role in the regulation of innate immune functions, such as the increase in ciliary beating (Lee et al., 2012; Shah et al., 2009). Interestingly, detection of bitter substances and bacterial quorum-sensing molecules from *P. aeruginosa* by the bitter taste receptor TAS2R38, which according to an immunostaining was located in ciliated cells, increased ciliary beat frequency via a mechanism involving NO (Lee et al., 2012, 2016). Similarly, in mouse nasal epithelial culture, *P. aeruginosa* quorum-sensing molecules induced a production of NO (Lee, Chen et al., 2014). The NO production was reduced in Trpm5$^{-/-}$ mice pointing towards the involvement of mouse nasal solitary chemosensory cells (Lee, Chen et al., 2014).

The importance of human TAS2R38 as a mediator of innate immunity in response to bacterial infections was underlined by the findings that polymorphisms in the encoding gene showed increased prevalence of sinonasal infections in patients (Lee et al., 2012). However, the presence of the bitter taste receptor TAS2R38 in human nasal ciliated cells is controversial, since in a recent study by Chen et al. (2019), TAS2R38 and TRPM5 expression were found exclusively in solitary chemosensory cells in human sinonasal biopsies. Upon a secondary analysis of the transcripts present in ciliated cells obtained in the single cell transcriptome study by Ordovas-Montanes et al. (2018), the authors confirmed their findings that taste signalling transcripts were not present in ciliated cells (Chen et al., 2019). The contradictory findings in humans might be due to different techniques used by Lee et al. (2012) and Chen et al. (2019), immunostaining *versus* analysis of RNAseq data combined with RNAscope *in situ* hybridization, respectively. Since problems with the specificity of antibodies are common, a validation of the labelling for TAS2R38, $\alpha$-gustducin, etc. in human epithelial cells, e.g. in cells deficient for the respective genes, would help to clarify this issue.

**Antimicrobial peptide secretion.** Activation of bitter taste receptors in human sinonasal cultures by *P. aeruginosa* metabolites or the bitter substance denatonium led also to the production of antimicrobial peptides, namely $\beta$-defensin 1 and $\beta$-defensin 2, and increased bacterial killing (Kohanski et al., 2021; Lee, Kofonow et al., 2014). In contrast, an activation of sweet taste receptors expressed in solitary chemosensory cells of mice and humans (Lee, Kofonow et al., 2014, 2017; Tizzano et al., 2011; Ualiyeva et al., 2020) by D-amino acids derived from gram-positive *Staphylococcus* suppressed the antimicrobial peptide secretion (Lee et al., 2017). Similar to human solitary chemosensory cells, D-amino acids were able to reduce the increase of $[Ca^{2+}]_i$ evoked by activation of bitter taste receptors in mouse nasal solitary chemosensory cells (Lee et al., 2017). This indicates that activation of taste receptors in nasal epithelia prompts protective innate immune responses as well as immune-suppressive effects. While it is clear that bitter taste receptor agonists induce an antimicrobial peptide secretion from human upper airway epithelial cells, it remains to be shown whether this was due to an activation of solitary chemosensory cells and/or other epithelial cell types. $\beta$-Defensin 2 secretion was dependent on $PLC_{\beta 2}$ pointing towards an involvement of solitary chemosensory cells. However, no involvement of Trpm5 in the antimicrobial effects to *P. aeruginosa* was detected for supernatants of human nasal epithelial cultures stimulated with denatonium (Lee, Kofonow et al., 2014). Interestingly, $\beta$-defensin production in response to stimulation of bitter taste receptors was exclusively found in human upper airway cultures and not observed in cultures derived from mouse nasal septum or in human bronchial epithelial cultures (Lee, Kofonow et al., 2014).

**Role of cysteinyl leukotrienes and ATP for solitary chemosensory cell function.** Some genes, such as the gene encoding the G protein-coupled receptor *Lgr5* associated with the Wnt pathway, are expressed at different levels in solitary chemosensory cells and brush cells. Mouse nasal $ChAT^+$ solitary chemosensory cells exhibit lower expression levels than tracheal $ChAT^+$ brush cells (Ualiyeva et al., 2020). The same study delineated two different solitary chemosensory cell populations in the nose that were both $ChAT^+$. One population expressed the genes *Il25*, *Il17rb* and *Egr2* to a greater extent while the other showed higher expression levels of *Gnat3* and *Dclk1*, suggesting that the two populations might be functionally different. Nevertheless, both subpopulations of nasal solitary chemosensory cells synthesise cysteinyl leukotrienes (Ualiyeva et al., 2020), indicating some common features of the two populations exist. Furthermore, aeroallergen-induced eosinophilia was not observed in *Pou2f3*$^{-/-}$ mice lacking all populations of chemosensory cells (Ualiyeva et al., 2020). The synthesis of cysteinyl leukotrienes can be induced by activation of the purinergic receptor P2Y2 in response to extracellular ATP (Ualiyeva et al., 2020). The most abundant purinergic receptor in nasal solitary chemosensory as well as in tracheal brush cells is the P2X4 receptor, whose function in these cells remains to be elucidated (Ualiyeva et al., 2020). Additionally, the source of extracellular ATP acting on solitary chemosensory cells needs to be determined. While there is currently no proof for a release of ATP from airway chemosensory cells, a release of ATP in response to the activation of type II taste cells, which are responsible for transmission of bitter, sweet and umami taste information in taste buds, is well established (Finger et al., 2005; Kinnamon & Finger, 2013).

**Transmission from chemosensory cells to nerve fibres.** In both upper and lower airways of mice, activation of chemosensory cells by bitter substances and bacterial quorum-sensing molecules led to an induction of neurogenic inflammation (Hollenhorst, Nandigama et al., 2022; Saunders et al., 2014) that was dependent on sensory nerve activation. The lower airways are innervated by sensory neurons located in the vagal jugular–nodose ganglia as well as in the thoracic dorsal root ganglia (Mazzone & Undem, 2016). The nose is innervated by sensory neurons originating from the trigeminal ganglion, as reviewed in more detail in Mazzone & Undem (2016). In the mouse lower airways, a decrease in the respiratory rate and an induction of protective breathing reflexes were observed after brush cell activation (Hollenhorst et al., 2020; Krasteva et al., 2012). In the upper airways, stimulation of the solitary chemosensory cells with bacterial quorum-sensing molecules evoked a decrease in the respiration rate due to a prolongation of the relative breath duration of the time between two breathing events. The reflex has been shown to be $G_\alpha$-gustducin- and Trpm5-dependent (Tizzano et al., 2010).

An activation of solitary chemosensory cells in the upper airways evoked a neurogenic inflammation, which was characterised by the release of the neuropeptide substance P from nerve fibres followed by a recruitment and degranulation of mast cells (Saunders et al., 2014). Binding of substance P to the neurokinin 1 receptor on mast cells seems to be crucial for the solitary chemosensory cell-induced neurogenic inflammation in the upper airways. In the lower airways mast cell recruitment and degranulation was only observed when bitter substances were applied at (supra)maximal doses, which activated sensory nerves directly. In contrast, brush cell-mediated responses (to low doses of agonists) were not accompanied by changes in mast cell number and activity (Hollenhorst, Nandigama et al., 2022). Further investigations are needed

for the definitive proof of mast cell contribution in the induction of neurogenic inflammation in the lower airways.

The role of CGRP released from nerve fibres in the upper airways for the induction of neurogenic inflammation was not addressed in the study by Saunders et al. (2014). In the lower airways, CGRP was shown to play a fundamental role in brush cell-induced neurogenic inflammation (see 'Neurogenic inflammation and protective response to bacterial infection'; Hollenhorst, Nandigama et al., 2022). Taken together, while in both upper and lower airways activation of chemosensory cells led to a protective neurogenic inflammation, the underlying mechanisms seem to differ.

## Microvillous cells in the olfactory epithelium

Different types of microvillous cells exist in the main olfactory epithelium (Hansen & Finger, 2008). Interestingly, certain populations of microvillous cells also express components of the bitter taste signalling cascade. Hansen & Finger (2008) found Trpm5 in two out of three different populations that were villin$^+$. Genovese & Tizzano (2018) identified PLC$_{\beta 2}$ in Trpm5$^+$ cells and showed expression of the sweet taste receptor T1R3 and $\alpha$-gustducin in microvillous cells as additional marker proteins for chemosensory cells. These cells are also cholinergic, since they express ChAT, yet the density of their innervating sensory nerve endings is contradictory (Genovese & Tizzano, 2018; Hegg et al., 2010; Ogura et al., 2011). Hegg et al. (2010) described a subset of microvillous cells innervated by substance P$^+$ fibres, while Ogura et al. (2011) and Genovese & Tizzano (2018) found only sparse innervation. The ChAT-expressing Trpm5$^+$ microvillous cells can be activated by bacterial lysates and cold saline (Ogura et al., 2011). Their neighbouring, supporting cells expressed muscarinic ACh receptors and can be activated by ACh. This led the authors to the conclusion that Trpm5$^+$ microvillous cells might sense important environmental stimuli and release ACh, which then acts on supporting cells (Ogura et al., 2011). Yet, this paracrine loop needs further experimental validation. Similar to chemosensory cells in other tissues, Trpm5$^+$ microvillous cells need the transcription factor Pou2f3 for proper development (Yamaguchi et al., 2014). They express transcripts of genes mediating inflammation and immune responses (*Il25*, *Il17rb*) as well as genes regulating viral entry in the host cells, viral transcription and genome replication (Baxter et al., 2021). Trpm5$^+$ microvillous cells react to ATP mainly via P2X receptors and some of them displayed spontaneous activity (Fu et al., 2018). Additionally, they can be activated by odour stimuli in primary cultures (Pyrski et al., 2017) and are necessary for odour-evoked responses, such as food detection, as shown by electro-olfactogram measurements and altered behaviour in mice in response to olfactory stimuli (Lemons et al., 2017). Additionally, microvillous cells are able to regulate the proliferation of basal stem cells as well as the apoptosis of cells in the dorsal recess of the main olfactory epithelium (Lemons et al., 2020). However, the functionality of the bitter taste signalling cascade in microvillous cells and their involvement in the induction of innate immune responses still needs to be shown.

## Tuft cells in the lung

Under healthy, physiological conditions chemosensory cells are only present in the upper and lower airways of human and mice, but not in the lung parenchyma. Recently, it was shown that after a viral influenza infection in mice, cells with characteristics of chemosensory cells, termed ectopic tuft cells by the authors, emerged in the lung (Rane et al., 2019). These lung ectopic tuft cells started to appear at day 12 post-infection and were detectable as late as day 49 post-infection. They derived from p63$^+$ intrapulmonary basal-like cells and were characterized by the expression of the brush cell and solitary chemosensory cell marker Dckl1, as well as of the members of the bitter taste signalling cascade Trpm5, Gnat3 and bitter taste receptors. Among the bitter taste receptors, the hallmark protein for tracheal brush cells, Tas2R108, was very prominent in the dysplastic regions of the lung of mice after influenza infection. Stimulation of lung ectopic tuft cells by denatonium or succinate induced plasma extravasation. This indicates that ectopic tuft cells in the alveolar region of the lung are functional chemosensory cells and most probably can trigger immune responses to aeroallergens and bacterial pathogens. In a follow up study, Barr and co-workers characterised these lung ectopic tuft cells on a transcriptional level and were able to delineate different tuft cell populations. The authors found the tuft-1, tuft-2 and the undifferentiated population described by Montoro and co-workers (see 'Brush cells in the lower airways') as well as a fourth population that was described as a 'stressed' tuft cell population (Barr et al., 2022; Montoro et al., 2018). The 'stressed' tuft cell population showed high expression of mitochondrial genes, such as *Aqp5*, *Hspb1*, *Selenbp1* and *Cbr2*. The tuft-1 cell population, known for the expression of taste-related genes, was identified by the marker genes *Gnb3* and *Ovol3*. The tuft-2 cell population, enriched in genes associated with leukotriene synthesis, was characterised by the expression of the marker genes *Alox5ap* and *Mgst3*. Notably, the post-influenza lung tuft cells, expressing chemosensory cell markers that can also be found in tracheal brush cells, showed lower levels of *Chat* and *Plcb2* when compared to

tracheal brush cells (Barr et al., 2022). While these lung tuft cells show the transcriptional profile of chemosensory cells and, thus, have been identified as members of the chemosensory cell family, their function and role in viral infections remains largely elusive. Since the tuft cells in the lung occur at a time point after viral clearance, they are obviously not needed for viral clearance itself. The appearance of these cells seems not to be necessary for tissue dysplasia after lung injury, since this phenomenon still occurred in tuft cell-deficient mice (Barr et al., 2022). Furthermore, they occurred independently of the type 2 cytokines, as concluded by the observation that they still appeared in *Il4ra*$^{-/-}$ and *Il25*$^{-/-}$ mice (Barr et al., 2022). Yet, the ectopic lung tuft cells seem to play an important role in orchestrating the immune response after viral infections, since mice lacking chemosensory cells in the airways that were infected with the PR8 influenza strain showed decreased macrophage recruitment as well as decreased expression of genes involved in chemotaxis (Melms et al., 2021). However, from this study it is not clear if this effect was due to lack of ectopic tuft cells in lung tissue or if this was due to a general loss of chemosensory cells in the airways that might lead to impaired communication between airways and lungs resulting in the reduced immune response.

A recent study with mice infected with the influenza A virus strain PR8 confirmed the increase of Dckl1$^{+}$ tuft cells in the lungs after infection (Roach et al., 2022). Interestingly, the authors also found an up-regulation of tuft cell numbers in the small intestine after influenza A infection. This increase in tuft cells in turn resulted in a tuft cell-dependent recruitment of ILC2s in the intestine, but not in the lungs. The authors speculated that this mechanism could be important to prevent or reduce systemic infections. Thus, the exact function of chemosensory cells in the lung after viral infections remains to be elucidated and might be rather important in preventing recurring infections rather than playing a role in acute infections, since they occur at a time point after viral clearance. Yet, the findings by Roach et al. (2022) provide intriguing new evidence for a communication between the respiratory tract and the intestine by a yet unidentified mechanism.

A recent study revealed that lung tuft cells occurred not only in the context of viral infection but also after treatment with several substances causing lung injury in mice, such as bleomycin and naphthalene (Huang et al., 2022), suggesting a broader impact of emerging tuft cells in pathophysiological conditions of the lung after diverse stimuli. These lung injury-evoked tuft cells were derived from ectopic basal cells via the WNT signalling pathway (Huang et al., 2022). However, about 20% of these ectopic tuft cells were derived from club cells (see also 'Brush cells in the lower airways'). The study by Huang et al. (2022) suggested that lung ectopic tuft cells had no impact on alveolar regeneration, since this was not altered in tuft cell-deficient *Pou2f3*$^{-/-}$ mice. Thus, the exact function of lung ectopic tuft cells that occur after lung injury remains to be elucidated.

The appearance of chemosensory cells in the lungs as a result of diverse pathological stimuli might also be relevant for humans. In the 1980s, two reports identified chemosensory cells in the lungs of children under pathological conditions by their morphology. In the first case this was a baby suffering from pneumonitis and in the second a 13-year-old person suffering from recurrent pneumonia and chronic bronchitis (DiMaio et al., 1988; Gordon & Kattan, 1984). Besides these early findings, a recent study provided evidence that expansion of chemosensory cells in response to viral infections also occurs in humans. Tuft-like cells that were positive for the chemosensory cell markers ChAT and POU2F3 were found in infected lung parenchyma of COVID-19 patients who died of COVID-19 (Melms et al., 2021). Additionally, the authors found an increase in the numbers of tuft-like cells in the upper airways of patients with fatal COVID-19. Interestingly, IL-13 treatment protects against SARS-CoV-2 virus and cell shedding. It was shown to affect viral entry, replication and cell-to-cell transmission and was associated with less severe COVID-19 (Morrison et al., 2022). Since IL-13 induces chemosensory cell expansion, it is tempting to speculate that the beneficial effects are at least partly due to the function of chemosensory cells.

## Conclusion

In summary, chemosensory cells in epithelia throughout the body have emerged as important therapeutic targets to stimulate the host's immune system. Unknowingly, we might already use bitter taste receptors in chemosensory cells to stimulate the immune system when prescribing antibiotics upon bacterial infections, since several antibiotics, such as levofloxacin, are able to activate taste receptors (Jaggupilli et al., 2019). Thus, it is tempting to speculate that antibiotics might also be involved in modulating the host immunity via the induction of protective innate immune responses upon activation of bitter taste receptors in chemosensory cells. While the role of antibiotics in this needs further investigation, chemosensory cells are able to respond to a wide variety of stimuli, including bacteria, viruses and protozoans, playing a crucial role in orchestrating protective immune responses.

Taken together chemosensory cells in the upper and lower airways seem to share more similarities than differences in eliciting innate immune functions, such as modulation of mucociliary clearance, recruitment of immune cells and induction of protective neurogenic

inflammation. Furthermore, studies from the nasal respiratory epithelium suggest that considerable similarities between mice and humans exist, making the mouse a suitable model for the translation of the research on chemosensory cells from animal to human. However, open questions remain on the exact mechanisms behind the chemosensory cell-induced immune responses, especially for longer lasting or chronic immune responses. It further remains to be elucidated whether it is possible that chemosensory cells also regulate the termination of the immune response when the infection is cleared. Additionally, the mechanisms that regulate the function of the chemosensory tuft cells that occur in response to viral infections need to be elucidated. Yet, the studies performed so far regarding airway chemosensory cells clearly demonstrate that these cells are key players in the cross talk between epithelia of the respiratory tract and the immune system.

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## Additional information

### Competing interests

The authors declare no competing interests.

### Author contributions

G.K.C. and M.I.H. conceived and wrote the article. Both authors approved the final version of the manuscript. Both authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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## Supporting information

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