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The Immune Function of Tuft Cells at Gut Mucosal Surfaces and Beyond

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Abstract

Tuft cells were first discovered in epithelial barriers decades ago, but their function remained unclear until recently. In the last two years, a series of studies has provided important advances that link tuft cells to infectious diseases and the host immune responses. Broadly, a model has emerged in which tuft cells use chemosensing to monitor their surroundings and translate environmental signals into effector functions that regulate immune responses in the underlying tissue. Here we review the current understanding of tuft cell immune function in the intestines, airways, and thymus. In particular, we discuss the role of tuft cells in type 2 immunity, norovirus infection, and thymocyte development. Despite recent advances, many fundamental questions about the function of tuft cells in immunity remain to be answered.

INTRODUCTION

Epithelial cells (and indeed many other non-hematopoietic cells) are perhaps underappreciated by immunologists who focus on cells of the hematopoietic system, yet they make crucial contributions to immunity. Most notably, epithelia form the body's barrier between self and non-self, and are therefore often the site of first encounter between the host and a foreign microbe or irritant. Although not as diverse as the hematopoietic compartment, epithelial barriers are comprised of multiple cell lineages with both overlapping and distinct functions. Goblet cells, for example, are professional mucus-producing cells, while Paneth cells secrete high levels of antimicrobial peptides, and enteroendocrine cells secrete hormones and communicate with the nervous system. The role of tuft cells, on the other hand, remained enigmatic for more than 60 years until a series of recent discoveries definitively linked tuft cells to immunity. In this review, we will focus on the immune function of tuft cells after a brief discussion of their development and markers.

CHARACTERISTICS & DISTRIBUTION

Tuft cells were first discovered in rat trachea (1) and mouse glandular stomach (2) in 1956, and in human trachea in 1959 (3). The advent of electron microscopy had allowed for visualization of cellular morphology in unprecedented detail, and several investigators

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quickly noted the presence of a rare but distinctive lineage of epithelial cells, which they termed tuft, brush, caveolated, multivesicular, or fibrillovesicular cells(1, 4). As these cells appear to be very closely related across tissues, we will refer to them collectively as tuft cells. Morphologically, tuft cells are characterized by 1) a “tuft” of long, blunt apical microvilli; 2) prominent actin, villin, and fimbrin rootlets that extend basally from the tips of the microvilli; and 3) abundant apical vesicles that form a tubulovesicular system. They are radiation-resistant epithelial cells (5) with a turnover rate equivalent to their surrounding epithelial cells, which is 3–5 days in the intestine (6–8) and 168–267 days in the trachea (9–11). With the exception of nascent tuft cells in intestinal crypts (12), tuft cells do not express the proliferation marker Ki67, indicating post-mitotic status in both the steady state (7, 12, 13) and during helminth infection (14).

In rodents, tuft cells have been identified in the digestive system [salivary glands (15), stomach (2), gall bladder and bile duct (16, 17), pancreatic duct (18), small intestine (19), cecum (20), and colon (21)]; the respiratory system [nasal cavity (22), auditory tube (23), and trachea (1)]; the urethra (24); and even in the thymus (25), a primary lymphoid organ. In rats, cells with tuft-like morphology have also been observed in alveolar epithelium (26), but in mice they have not been seen below the bronchial branch point. In humans, cell with tuft-like morphology were reported in the trachea (3), small intestine (27, 28), stomach (29, 30), gallbladder (31), and in the alveoli of a 4-month-old patient with pneumonitis (32). As a rule, tuft cells are found in hollow organs or tubes lined by a non-squamous epithelium, but the thymus is a notable exception and there are non-squamous mucosal barriers where tuft cells have as yet not been described, such as the female reproductive tract.

LINEAGE SPECIFICATION

Although tuft cells are found in many tissues, their development and lineage specification has only been studied in detail in the small intestine, likely because the stem cells of the intestinal epithelium are among the best characterized and most prolific in the body (33). In homeostasis, these cells reside at the base of intestinal crypts, express the marker LGR5[2], and produce enough progeny to replace the entire intestinal epithelium in just 3–5 days (6). Lineage tracing has demonstrated that intestinal tuft cells are indeed derived from $LGR5^+$ stem cells (7), but unlike all other epithelial cells, differentiated intestinal tuft cells continue to express *Lgr5* (34, 35).

Immediately above the $LGR5^+$ stem cell compartment is the transit amplifying zone, where uncommitted epithelial progenitors replicate and adopt their terminal fate. The first lineage branch point is regulated by a classical lateral inhibition model in which cells receiving a Notch signal upregulate Hairy and enhancer of split-1 (*Hes1)* and become enterocytes (36), while those providing a Notch ligand (i.e. Delta-like-ligand 1 (DLL1)-expressing progenitors) retain potential to become all non-enterocyte lineages (goblet, enteroendocrine, Paneth, and tuft). Loss of Notch signaling induces the transcription factor *Atoh1*[3], which goblet, enteroendocrine, and Paneth cells all constitutively express. Accordingly, these cells

[2]Lgr5: Leucine-rich repeat-containing G-protein coupled receptor 5
[3]Atoh1: Atonal bHLH Transcription Factor 1

are absent when *Atoh1* is deleted from epithelial stem cells (7, 12, 37). Mature tuft cells, on the other hand, do not express *Atoh1*, and studies that deleted *Atoh1* from all intestinal epithelial cells reported conflicting results about the requirement of *Atoh1* in intestinal tuft cell development. While tuft cells were absent in the small intestine of Villin-Cre^{Ert2} X $Atoh1^{f/f}$ mice (7), their numbers were normal or even increased in Rosa26-Cre^{Ert2} X $Atoh1^{f/f}$ (12), Lgr5-Cre^{Ert2} x $Atoh1^{f/f}$ (38), and Lrig-Cre^{Ert2} X $Atoh1^{f/f}$ (37) mice. Interestingly, colonic tuft cells, which were only studied in Lrig-Cre^{Ert2} X $Atoh1^{f/f}$ mice, did require *Atoh1,* suggesting distinct mechanisms of lineage specification in the small intestine and colon. Although some uncertainty remains, on balance these studies suggest that a binary HES1/ATOH1 model does not fully explain the differentiation of intestinal epithelium.

Intestinal tuft cell development is not affected in the absence of the transcription factors neurogenin 3 (NEUROG3), SAM pointed domain containing ETS transcription factor (SPDEF), and Sex-determining region Y-box 9 (SOX9), which are the lineage transcription factors that define enteroendocrine cells, goblet cells and Paneth cells (7, 12). Instead, POU2F3[4] and GFI1B[5] have been suggested as tuft cell-specific master regulators. All tuft cells express both markers constitutively (12, 39, 40), and tuft cells are entirely absent in *Pou2f3*$^{-/-}$ mice, while all other epithelial lineages appear normal, at least in the intestine (39). The status of tuft cells in the absence of *Gfi1b* has not been reported and it is unknown if either POU2F3 or GFI1B is sufficient to drive tuft cell differentiation. In the airway, tuft cells were shown to be derived from basal cells with rapid kinetics in lineage tracing experiments, but the precise signals that specify the tuft cell lineage remain uncertain here as well(41).

The timing of tuft cell emergence during development also remains unclear. One study found that tuft cells appeared by mouse embryonic day 18.5(E18.5) in developing intestine and stomach antrum (30); in other studies, tuft cells did not appear until after birth (42, 43). These studies all demonstrated that both gastric and intestinal tuft cell frequency remained very low before reaching adult-equivalent density after weaning (30, 42, 43), with similar frequency between small intestine and colon in unmanipulated mice (35). In contrast, tracheal tuft cells are present at adult frequency by at least day 5 post-birth (11). Overall there is much more to be learned about tuft cell lineage specification across diverse tissues, particularly how it can be regulated by immune signals in homeostasis and diseases. IL-13 in small intestinal stem cells, for example, can induce tuft cell hyperplasia (14, 44 and discussed in detail below), but it is unclear if this occurs in any other tissues.

TUFT CELL HETEROGENEITY

Although morphologically very similar, the developmental and functional equivalence of tuft cells in different tissues and even in different regions of the same tissue remains unclear. A list of tuft cell markers is included in Table 1 and we recently used bulk RNA-sequencing of Epithelial cell adhesion molecule $(EPCAM)^+$ IL-25^+ tuft cells from five different tissues to

[4]Pou2f3: POU class 2 homeobox 3
[5]Gfi1B: Growth factor independent 1B

identify a core transcriptional signature that is shared by all tuft cells (45). In addition to *Il25*, this signature includes many of the markers listed in Table 1, such as *Dclk1*[6], *Trpm5*[7], *Prostaglandin endoperoxide synthase-1 (Ptgs1), Pou2f3*, *Gfi1b*, and *Sialic acid binding Ig-like lectin F* (*Siglecf)*. Despite these shared features, RNA sequencing as well as immunostaining have also revealed significant inter- and intra-tissue diversity of tuft cells. For example, a recent study used multiplex immunofluorescence to demonstrate that individual DCLK1+ tuft cells in the intestine express differential levels of markers such as acetylated tubulin (acTUB), SOX9, PTGS1 (COX1) and PTGS2 (COX2) (35). Tuft cell heterogeneity was also identified using single-cell sequencing, which led to the classification of Tuft-1 and Tuft-2 subsets in both the airway and intestine (46, 47). In both tissues, expression of eicosanoid biosynthesis genes and *Ptprc* (a.k.a. *Cd45*) is enriched in Tuft-2 cells. Intestinal Tuft-1 cells express a neuronal signature, while the tracheal Tuft-1 subset is associated with a taste transduction signature. In terms of cytokines, *Il25* is constitutively expressed in all tuft cells, while *Tslp* is detectable in both Tuft-1 and Tuft-2 cells of the trachea but only in Tuft-2 cells of the small intestine. In the trachea there is also subset-specific skewing of transcription factors, with Tuft-1 cells enriched for *Pou2f3* and Tuft-2 cells for *Gfi1b*, but both genes remain detectable in all tuft cells. By bulk RNA sequencing (45), one key distinction between tuft cells from distinct tissues was the differential expression of surface receptors, suggesting that tuft cells have evolved to sense different ligands depending on their microenvironment. Whether the effector functions of tuft cells are also tissue-specific requires further investigation.

IMMUNE FUNCTION OF TUFT CELLS IN THE INTESTINE

Tuft-ILC2 Immune Circuit

The initiation of type 1 immune responses, from innate immune sensing to priming of adaptive cells, is relatively well understood. By contrast, much less is known about how helminths, protists, and allergens trigger a type 2 response. Group 2 innate lymphoid cells (ILC2s) are the dominant early source of IL-5, IL-9, and IL-13 in numerous models of type 2 inflammation (48–52), and understanding how ILC2s are activated has therefore been of great interest. ILC2s lack an antigen receptor and there is little evidence that they sense type 2 agonists directly. Instead, ILC2s integrate numerous host-derived activating signals, including cytokines (e.g., IL-33, IL-25) (48, 53, 54), lipids (e.g., leukotrienes) (55–57), and neuronal peptides (e.g., Vasoactive intestinal peptide (VIP), Neuromedin U (NMU)) (49, 58–61). Current models propose that ILC2s use these signals to monitor the status of their surrounding tissue and become activated by disruptions in homeostasis (62).

In the intestine, the link between IL-25 and helminth-induced type 2 responses is well-established: IL-25 (63, 64, 54) and its downstream adaptor Act1 mediate type 2 immunity to promote worm expulsion (65). Furthermore, IL-25 is sufficient to activate ILC2s and promote worm expulsion independently of adaptive Th2 function (48, 64). But the physiologic cellular source of IL-25 remained elusive until recently. Using *Il25*-RFP reporter mice and immunohistochemistry, recent studies identified tuft cells as the dominant source

[6]Dclk1: Doublecortin-like kinase 1
[7]Trpm5: Transient receptor potential cation channel subfamily M member 5c

of IL-25 in the small intestine both at homeostasis and during helminth infection (14, 39). Tuft cell-derived IL-25 helps to drive a feed-forward tuft-ILC2 signaling circuit in which ILC2s are activated to produce IL-5, −9, and −13, thereby promoting type 2 inflammation. IL-13 also signals in undifferentiated epithelial cells, skewing their lineage commitment towards tuft and goblet cells (14, 39, 44). Due to the rapid turnover of the intestinal epithelium, activation of the tuft-ILC2 circuit quickly results in pronounced tuft and goblet cell hyperplasia. The frequency of tuft cells, in particular, can increase more than 10-fold during helminth infection. This circuit can be activated exogenously by stimulating ILC2s with recombinant IL-25 or IL-33, or by giving recombinant IL-13 to drive tuft cell hyperplasia directly in the intestinal epithelium. Removing components of the tuft-ILC2 circuit (e.g *Pou2f3*$^{-/-}$, *Il25*$^{-/-}$, and *Il4Ra*$^{-/-}$) disrupts the intestinal type 2 response and leads to delayed clearance of the roundworm *Nippostrongylus brasiliensis* (14, 39, 66). Conversely, deleting the innate immune signaling inhibitor TNF alpha induced protein 3 (*Tnfaip3*, encoding A20) from ILC2s leads to chronic activation of the small intestinal tuft-ILC2 circuit driven by the constitutive expression of IL-25 in tuft cells (43)

Tuft cells are also found constitutively in the gall bladder, pancreatic ducts, cecum, and colon, where they express many of the same markers (e.g. DCLK1, CHAT[8], TRPM5) as in the small intestine. An immune function has not, however, been reported for any of these cells. In fact, all evidence so far suggests that the tuft-ILC2 circuit does not operate in these tissues. For example, deleting A20 from ILC2s spontaneously activates the tuft-ILC2 circuit in the small intestine, but there is no evidence of type 2 inflammation in any other intestinal tissues (43). Further, systemic delivery of recombinant IL-4 drives tuft cell hyperplasia only in the small intestine (von Moltke & Locksley, unpublished). It may be that tuft cells and ILC2s still communicate outside the small intestine, but that IL-4/13 signaling in epithelial stem cells at these sites does not induce tuft cell hyperplasia. Small and transient changes in tuft cell frequency have been noted in the colon when germ-free mice are colonized with bacteria (35), but the mechanism for these fluctuations remains unknown.

In young, unmanipulated mice, ILC2s are the dominant IL-25 receptor-expressing and IL-13-producing tissue-resident cells, and are therefore critical for rapid (7–10 days) clearance of the rodent roundworm *N. brasiliensis*. In chronic infection settings (e.g. with the helminth *Heligmosomoides polygyrus*) or once immune memory is established, other sources of IL-13 are activated and can likely substitute for ILC2s in the circuit. In fact, the connections between tuft cells and adaptive immunity remain completely unexplored. In addition, several details of the innate tuft-ILC2 circuit require further examination. In particular, how is the circuit regulated if IL-25 expression is constitutive, and what do tuft cells do besides secrete IL-25? Besides cytokines, tuft cells also express enzymes for eicosanoid biosynthesis, such as *Cox-1*, *Cox-2*, 5-lipoxygenase (*Alox5*), and hematopoietic prostaglandin-D synthase (*Hpgd*) (67, 7, 68, 69, 14, 46). How eicosanoid biosynthesis is regulated in tuft cells and the physiologic function of tuft cell-derived eicosanoids remain unknown.

[8]CHAT: choline acetyltransferase

Tuft Cell Chemosensing

Soon after the link between tuft cells and helminth infection was established, another landmark study revealed that the tuft-ILC2 circuit is also activated by intestinal colonization with *Tritrichomonas*, a genus of protists found in the commensal flora of many mouse vivariums (44). This study also provided the first functional evidence of a link between chemosensing by tuft cells and type 2 immunity.

Immune cells and intestinal epithelial cells are known to sense microbially-derived molecular patterns with pattern recognition receptor (PRRs) such as Toll-like receptors to initiate type 1 immunity, but the molecular stimuli and the cell type(s) that drive type 2 responses are still elusive. A sensing function has long been hypothesized for tuft cells, and immunostaining provided the first clues that a chemosensing pathway previously characterized in taste transduction might also be active in tuft cells (70–72, 68). In taste receptor cells, signaling through canonical G protein-coupled taste receptors activates a specialized G alpha subunit known as alpha-gustducin (GNAT3), which in turn initiates intracellular calcium flux via phospholipase C beta 2 (PLCB2) (73). The rise in Ca^{2+} opens the cell surface cation channel TRPM5, leading to depolarization of taste cells. When the first complete transcriptome analysis of intestinal tuft cells was completed in 2008, it confirmed that all components of the pathway, except canonical taste receptors, are indeed highly and selectively expressed in tuft cells (67).

Tuft cells are ideally positioned to act as immune sentinels by monitoring the intestinal lumen and transmitting signals to immune cells in the underlying tissue. Howitt et al. provided the first direct evidence for such a function, by demonstrating that *Trpm5*$^{-/-}$ and *Gnat3*$^{-/-}$ mice fail to induce tuft cell hyperplasia when colonized with *Tritrichomonas* (44). Immune responses to the helminths *N. brasiliensis* and *H. polygyrus* are also impaired in *Trpm5*$^{-/-}$ mice(44), but tuft cell hyperplasia occurs normally in *Gnat3*$^{-/-}$ mice colonized with *N. brasiliensis*, suggesting distinct sensing mechanisms for helminths and protists (45).

The lack of canonical taste receptor expression in intestinal tuft cells suggested the hypothesis that other G-protein coupled receptor(s) (GPCR) may be specifically enriched on tuft cells to ‘sense’ protists and helminths. Indeed, the extracellular succinate receptor 1 (SUCNR1) was recently identified to be selectively expressed in both TRPM5+ and IL-25+ small intestinal tuft cells (45, 67, 74). Remarkably, providing succinate in the drinking water of mice is sufficient to drive tuft cell hyperplasia in a *Sucnr1*-, *Il25*- and *Trpm5*-dependent manner (45, 74). Succinate treatment also induces other hallmarks of type 2 responses, such as goblet cell hyperplasia, eosinophilia, and IL-13 production by ILC2s (43, 45). Further, the activation of ILC2s by succinate is *Il25*-, *Trpm5*-, and *Pou2f3*-dependent (43, 45, 74). Succinate is therefore the first ligand identified for intestinal tuft cells and one of the only known innate immune ligands that is sufficient to activate type 2 inflammation.

Succinate is an intermediate of the citric acid cycle and is normally sequestered inside host cells. Many microbial pathogens and commensals, on the other hand, have evolved diverse fermentative metabolic pathways to thrive in the nutrient-rich but oxygen-poor intestinal lumen, and these pathways frequently result in production and secretion of succinate (75). Succinate is detectable in the supernatants of *in vitro* cultured *N. brasiliensis* and

Tritrichomonas and in the cecum of mice monocolonized with *Tritrichomonas*. Accordingly, the detection of *Tritrichomonas* by tuft cells is entirely SUCNR1-dependent (43, 45). By contrast, the immune response to *N. brasiliensis* is intact in *Sucnr1*$^{-/-}$ mice (45, 74), demonstrating that SUCNR1 signaling is absent or redundant during helminth infection and underscoring the differences between sensing of protists and helminths that was suggested by experiments using *Gnat3*$^{-/-}$ mice. There is also evidence that bacterial dysbiosis leads to SUCNR1-dependent tuft cell hyperplasia, although it is not clear whether this occurs physiologically (74). Together, these studies identify a specific metabolite that selectively activates the tuft-ILC2 circuit and define a paradigm in which the intestinal type 2 immune system monitors microbial metabolism. Tuft cells also express another potential metabolite sensor –the short chain fatty acid receptor *Ffar3* (45, 46)— but a function for this receptor has remained elusive.

The seemingly intact immune response to *N. brasiliensis* in *Gnat3*$^{-/-}$ and *Sucnr1*$^{-/-}$ mice suggests that there is at least one other sensor upstream of TRPM5 that detects helminth infection. There are also questions remaining about the mechanisms of chemosensing by tuft cells in other tissues. The detection of succinate also warrants further investigation. In particular, the benefits of sensing *Tritrichomonas*-derived succinate are not clear, since these protists are not eliminated or even reduced in number by the type 2 immune response(45). Given that most protists and helminths have evolved to establish chronic colonization, their sensing by the immune system may therefore be linked principally to host adaptation and tolerance. In support of this idea, activation of the tuft-ILC2 circuit was recently shown to drive small intestinal lengthening (43). Since tuft and goblet cell hyperplasia lead to a decreased frequency of absorptive enterocytes, this intestinal lengthening may help to maintain the absorptive capacity of the intestine. Indeed, there is no overt loss of fitness or decrease in caloric uptake associated with chronic activation of the tuft-ILC2 circuit (43).

Tuft Cells and Norovirus

Human norovirus is the leading cause of gastroenteritis outbreaks worldwide and the acute phase of disease can be followed by weeks or months of viral shedding in the stool (76), suggesting a site of viral persistence in the host. Murine norovirus (MNoV) is even more persistent, with some strains establishing chronic infection, but the cellular tropism *in vivo* remained unclear until recently. In 2017, immunostaining of non-structural norovirus proteins demonstrated that a rare population of EPCAM^{+} cells in the small intestine and colon serve as the exclusive viral reservoir in mice infected with MNoV^{CR6} (77). These cells were soon identified to be tuft cells, which express high levels of the MNoV receptor CD300LF (5). Accordingly, mice were resistant to infection with MNoV^{CR6} when tuft cells were absent or decreased, while viral titers were enhanced in any context where tuft cell numbers were increased, such as helminth infection or treatment with recombinant IL-25. In contrast, the non-persistent strain MNoV^{CW3} was unable to infect intestinal epithelial cells (5, 77). Whether human norovirus and/or other enteric viruses also infect tuft cells remains to be determined. It also remains unclear why norovirus would target tuft cells for infections. Perhaps the unique cell biology of tuft cells is important for viral replication, or tuft cells represent an immune-privileged site.

IMMUNE FUNCTION BEYOND THE INTESTINE

Airway Tuft Cells

Although often referred to as brush cells, a population of airway epithelial cells has been identified in the murine and human trachea that share the unique morphology and transcriptional signature of intestinal tuft cells(10, 41, 45, 78). Another very closely related cellular lineage termed solitary chemosensory cells (SCC) has also been identified in the nasal epithelium of mice and humans(22, 72, 79, 80), but its precise relationship to tuft cells has not yet been established. Unlike intestinal tuft cells, both tuft cells and SCCs of the airways express type II taste receptors (T2Rs, also known as bitter taste receptors) in humans (79) and mice (22, 81), and bitter taste receptor polymorphisms correlate with gram-negative bacterial infection in humans (82). T2Rs have been linked to regulation of both tissue physiology and immune responses. For example, denatonium—a potent bitter taste receptor ligand—can act on tuft cells to regulate respiration rate (83), nasal neurogenic inflammation (84), and allergic asthma induced by ovalbumin (OVA) and house dust mite (HDM) in mice (85). The bitter receptors on SCCs are reported to detect acyl-homoserine lactones (AHLs), which are produced by gram-negative bacteria (e.g. *Pseudomonas aeruginosa*) (86) to indicate population density (80, 87). Moreover, bitter taste receptors activate calcium flux in nasal SCCs to stimulate anti-microbial peptide secretion from surrounding epithelial cells and promote killing of *P. aeruginosa*, methicillin-resistant *S. aureus* (MRSA), *K. pneumonia*, and *S. epidermis* in human sinonasal tissue (80, 88). SCCs also express canonical sweet taste receptors (T1R2/3), but their activation suppresses calcium flux and bitter taste receptor-induced antimicrobial responses(88). T1R2/3 mediate sensing of glucose and bacterial D-amino acids in SCCs, leading to reduced antimicrobial peptide secretion (β-defensin) (88, 89). Clinically, there are elevated glucose and amino acid concentrations in chronic rhinosinositis patients and colonized fibrosis patients, respectively (88, 90). Together, these important studies suggest that SCCs, and perhaps airway tuft cells, utilize chemosensory machinery to ‘taste’ the upper respiratory tract environment and regulate innate immunity.

Whether airway tuft cells and SCCs are also integrated into tuft-ILC2 circuits and how this might alter type 2 immune responses remains unknown. Manipulation of IL-25 by intranasal administration, systemic blockade, or genetic deficiency all regulates lung type 2 airway inflammation (91, 64, 92, 93), but the expression of IL-17RB (a subunit of the IL-25 receptor) is much lower on lung ILC2s than in the intestine, even with IL-25 injection (94). Furthermore, the restriction of tuft cells to the upper airways in mice is confounding when considering inflammatory responses in the distal lung (10). In humans, SCCs are the major source of IL-25 in patients with chronic rhinosinusitis (95, 96), and ILC2 numbers were elevated in nasal polyps from chronic rhinosinusitis patients (96). These results support the existence of a tuft-ILC2 circuit in the human nasal cavity. There were also human case reports suggesting that brush (tuft) cell numbers are altered in immotile cilia syndrome (97) and interstitial pneumonitis (32). Together, future studies may further investigate the expression, detection mechanism, and corresponding immune function of taste receptors and other novel G-protein receptors on airway tuft cells, especially in the upper versus lower respiratory tract.

Thymic Tuft Cells

In all of the examples discussed so far, tuft cells were found in the non-squamous epithelium of hollow tissues. Therefore, it was surprising when cells with tuft-like morphology and expression of *Gnat3, phospholipase C beta 2 (Plcb2), and Chat* were identified in the thymic medulla, although unlike tuft cells in upper airways, no contact of these cholinergic chemosensory cells with nerve fibers was observed (25). More recently, RNA sequencing and careful phenotyping confirmed that these cells are indeed bona fide tuft cells and that they comprise 3~10% of medullary thymic epithelial (mTEC) cells in murine thymus and ~3.5% of mTEC in human thymus (98, 99). Based on single cell sequencing, tuft cells comprise mTEC group IV, which is molecularly distinct from other mTECs but closely related to intestinal tuft cells, with *Dclk1*, *Sox9*, *Trpm5*, *Il25* and *Pou2f3* all being expressed (99, 100). There are, however, also important differences between thymic tuft cells and those of other tissues; most notably, IL-25^{+} thymic tuft cells express MHC-II, suggesting an antigen presenting function (99). Thymic tuft cells also express a diversity of canonical taste receptors, which have been described in airway tuft cells but appear to be largely absent in intestinal tuft cells (45). As in other tissues, thymic tuft cell development requires *Pou2f3* (99, 100).

Functionally, thymic tuft cells support TCRβ^{int} CD1d^{+} IL-4^{+} invariant NKT2 thymocytes and EOMES^{+} CD8 thymocytes in an *Il25-, Pou2f3-*, and *Trpm5*-dependent manner, although why chemosensing would be required for this function is completely unclear (99). The frequency of thymic Lin^{-} TCR^{-} CD127^{+} GATA3^{+} ILC2s is increased in the absence of tuft cells, but the functional significance of this remains unknown (100). Neither *Pou2f3* nor *Trpm5* deficiency impacted CD4^{-}CD8^{-}, CD4^{+}CD8^{+}, CD4 single positive T cells (CD4SP), or CD8SP numbers in the thymus (98, 99). In sum, thymic tuft cells are a distinct population of mTECs that regulate the frequency of certain thymocyte subsets. Their ‘sensing’ mechanism by taste receptors, their ontogeny, their relationship to antigen presentation, and their function in immune tolerance remain enigmatic.

TUFT CELLS AND NEURONS?

In addition to the outstanding questions already highlighted throughout this review, there is significant interest in the possibility that tuft cells might communicate with neurons, a finding which could provide mechanistic insight for numerous recent studies that have broadly linked the nervous and immune systems and specifically implicated neuronal signaling in type 2 inflammation (58, 101). In this context, it is notable that tuft cells in all tissues express CHAT (83, 68, 84, 45), the enzyme required for synthesis of the neurotransmitter acetylcholine. To date, a link between tuft cells and neurons has been best characterized in the airway. Although tuft cells and neurons do not form synaptic connections, they have been imaged in close proximity in the airway (102, 83, 84), and some of those neurons express acetylcholine receptors (84, 103). Accordingly, the airway inflammation induced by stimulating SCCs requires *Trpm5* and acetylcholine (84). For the most part, however, tuft-neuron interactions have not been linked directly to immunity. For example, changes in respiratory rate induced by bitter substances are absent in *Trpm5*$^{-/-}$ mice and in mice where the airway epithelium has been abraded, suggesting that

chemosensing by tuft cells regulates smooth muscle activity (80, 83), presumably via neuronal signaling. Similarly, bitter substances induce acetylcholine release from urethral tuft cells and cause contraction of the bladder detrusor muscle when delivered *in vivo* (104). Whether these tuft-neuron interactions in the airway and urethra represent mechanisms of avoidance and/or flushing that provide immune protection has not been tested.

The link between tuft cells and neurons in the intestine remains much less clear given different findings regarding tuft-neuron proximity (68, 71, 105). Intriguingly, intestinal tuft cells are positive for both CHAT and the neuropeptide β-endorphin (7, 68), and co-culture of neurons and intestinal organoids supports the differentiation of tuft cells (38). Interactions between tuft cells and the enteric nervous system, if verified, might serve to expand the sensing capacity of the nervous system while also broadly distributing signals initiated in tuft cells.

CONCLUSIONS

After decades of pioneering work provided the first hints of a chemosensing pathway in tuft cells and suggested a role for tuft cells in response to bacterial colonization in the airways, the last two years brought a series of breakthroughs that definitively implicated tuft cells in immune sensing and regulation. It is now clear that tuft cells are a critical component of the type 2 immune response, provide a reservoir for chronic norovirus infection, and contribute to thymic function. While it has been exciting to find an immune role for the previously enigmatic tuft cell lineage, we speculate that this does not represent their most evolutionarily ancient function. Undoubtedly, tuft cells have been critically shaped by co-evolution with helminths, protists, norovirus, and perhaps other microbes, but just as goblet cells produce mucus at homeostasis to support epithelial function and can be hyper-activated to promote helminth expulsion, we propose that tuft cells first evolved epithelial effector functions that were later useful for immunity and/or pathogenic exploitation. In this context, it is intriguing that tuft cells have been implicated in airway contraction, epithelial regeneration, DNA damage repair (18, 69, 106), and tumorigenesis (38, 107–109). If correct, our hypothesis suggests that understanding the unique cell biology of tuft cells and their role in the absence of infection will also advance our understanding of tuft cells in immunity.

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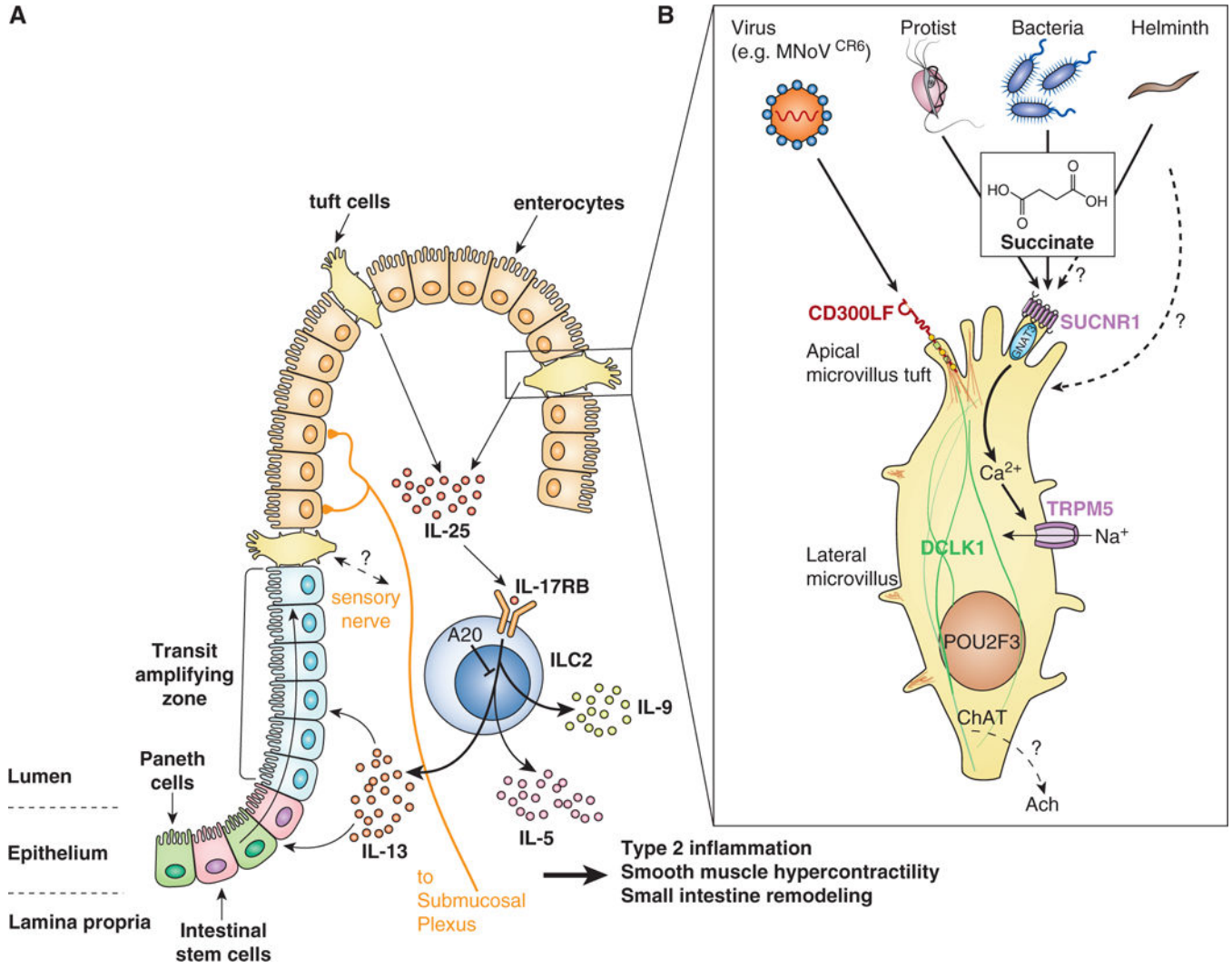

Figure 1: Chemosensing by intestinal tuft cells regulates the tuft-ILC2 circuit

(A) **Intestinal Tuft-ILC2 circuit.** Tuft cells constitutively express *Il25*, which acts on group 2 innate lymphoid cells (ILC2s) in the lamina propria to induce production of canonical type 2 cytokines IL-5, −9, and −13, which collectively drive all aspects of innate type 2 inflammation, including eosinophilia and intestinal remodeling. IL-13 in particular signals in undifferentiated epithelial cells to bias their lineage commitment towards tuft and goblet cells, leading to hyperplasia of both cell types and driving the feed-forward tuft-ILC2 circuit. The circuit is amplified but yet unknown mechanisms when helminths or protists are sensed by tuft cells. Deletion of the signaling repressor A20 from ILC2s also amplifies the circuit and leads to chronic type 2 inflammation in the small intestine.

(B) **Chemosensing.** Tuft cells sense succinate secreted from *Tritrichomonas* protists and perhaps also bacteria and helminths. Signaling through the G protein coupled succinate receptor SUCNR1 induces an intracellular Ca^{2+} flux that opens the cation channel TRPM5, leading to influx of Na^{+} and depolarization of the cells. How cellular depolarization regulates tuft cell effector functions remains unknown, but may include release of neurotransmitter (e.g. acetylcholine (ACh)) that acts on nearby neurons. Tuft cells also

express the murine norovirus receptor CD300LF and are the host reservoir for chronic infection by the CR6 strain of norovirus. It is not clear if and how tuft cells sense this infection.

Table 1:

Tuft cell biomarkers. Markers were grouped by their functional role in tuft cells (ex: structural, chemosensing..etc)

Structural Markers		
Marker	**Description**	**Comment**
DCLK1	Doublecortin like kinase 1 is a microtubule-associated kinase first described in neurons that regulates polarization (103).	The most widely used tuft cell marker. Tuft cells in all tissues express DCLK1 and >95% of DCLK1+ epithelial cells in the murine intestine are tuft cells
VIL1	Villin1 is an actin-binding protein that is abundant in microvilli and therefore concentrated at apical tip of tuft cells (104) (105, 106).	Apical concentration of VIL1 is unique to tuft cells, but all intestinal epithelial cells express *Vil1*. Since Vil1-Cre is widely used to target the intestinal epithelium, it is worth noting that tuft cells in respiratory tract also express *Vil1* (25).
acTUB	Acetylated-alpha-tubulin is required to form microtubule bundles, which are abundant in tuft cells (6, 29).	Highly specific marker for tuft cells but not widely used.
CK18	Cytokeratin 18 colocalizes with villin in tuft cells (25, 107).	Some evidence of non-tuft CK18^{lo} epithelial cells (11, 107).
UEA-1	Ulex europaeus agglutinin type 1 is an abundant lectin on the apical surface of intestinal tuft cells (11, 108).	Although relatively selective for tuft cells in the proximal small intestine, in the distal intestine UEA-1 is widespread on all epithelial cells.
Chemosensing Markers		
Marker	**Description**	**Comment**
TRPM5	Transient receptor potential cation channel subfamily M member 5 is a calcium-gated cation channel thought to regulate depolarization upon chemosensory stimuli (109)	Based on TRPM5-GFP reporter, all tuft cells in intestine (64, 69) and nasal cavity (76, 81) express TRPM5.
GNAT3	Alpha-gustducin is a specialized G protein that couples to canonical taste receptors and perhaps other 7 pass transmembrane receptors as well.	GNAT3 has been detected in non-tuft epithelial cells in the airway (110, 111), and genetic experiments have demonstrated that unlike TRPM5, not all tuft cell sensing is GNAT3-dependent(42)
PLCB2	Phospholipase C beta 2 is activated downstream of GNAT3.	PLCB2 has been detected in non-tuft epithelial cells in the airway (110, 111).
CHAT	Choline acetyltransferase catalyzes the formation of the neurotransmitter acetylcholine	Based on Chat-GFP reporter and antibody staining, tuft cells in airways(77), gastro-intestinal tract(64, 65, 98), urethral tract(23, 97), and thymus(24) express *Chat*, but some *Chat* negative tuft cells have also been observed(65).
Transcription Factors		
Marker	**Description**	**Comment**
POU2F3	POU class 2 homeobox 3 was first identified as being required for differentiation of taste receptor cells (112), but *Pou2f3*$^{-/-}$ mice are also completely tuft cell-deficient	POU2F3 constitutively expressed in all tuft cells, including early tuft cells in intestinal crypts (38, 39).
GFI1B	Growth factor independent 1B is a transcriptional repressor.	GFI1B is constitutively expressed in all tuft cells, including early tuft cells in intestinal crypts (11, 13, 38). It may also expressed in M cells(113). Its function in tuft cells is unknown.

Structural Markers		
Marker	**Description**	**Comment**
Other		
Marker	**Description**	**Comment**
IL-25	Interleukin 25 is associated with type 2 inflammation and is required for intestinal clearance of helminths.	Tuft cells in all tissues analyzed constitutively express IL-25 and all DCLK1+ cells in the intestinal epithelium are also IL-25+ (13)
PTGS1	Prostaglandin-endoperoxide synthase 1 (a.k.a. COX-1) is required for synthesis of cyclooxygenases	All tuft cells appear to express PTGS1 and tuft cells are the only epithelial cells in the intestine that express this enzyme (11, 13, 38, 64)
SIGLECF	Sialic acid binding Ig-like lectin F encodes immunoreceptor tyrosine-based inhibnitory motifs and is normally associated with hematopoietic lineages (e.g. eosinophils).	Cell surface marker that can be used in flow cytometry(63). Its function in tuft cells is unknown
p-EGFR	EGFR phosphorylated on tyrosine 1068 (34).	Only tested in intestine